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(54) Title: SPIDER SILK PROTEIN ENCODING NUCLEIC ACIDS, POLYPEPTIDES, ANTIBODIES AND METHOD OF USE THEREOF

(57) Abstract: Spider silk protein encoding nucleic acids, polypeptides and antibodies immunologically specific therefore are disclosed. Methods of use thereof are also provided.

SPIDER SILK PROTEIN ENCODING NUCLEIC ACIDS, POLYPEPTIDES, ANTIBODIES AND METHODS OF USE THEREOF

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FIELD OF THE INVENTION

This invention relates to the fields of molecular and cellular biology. Specifically, nucleic acids encoding spider silk polypeptides, spider silk polypeptides, spider silk polypeptide-specific antibodies, and methods of use thereof are provided.

BACKGROUND OF THE INVENTION

Several publications are referenced in this application by numerals in parentheses in order to more fully describe the state of the art to which this invention pertains. Full citations for these references are found at the end of the specification. The disclosure of each of these publications is incorporated by reference herein.

Spider silks comprise a model system for exploring the relationship between the amino acid composition of a protein, the structural properties that result from variations in the amino acid composition of a protein, and how such variations impact protein function. While

silk production has evolved multiple times within arthropods, silk use is most highly developed in spiders. Spiders are unique in their lifelong ability to spin an array of different silk proteins (or fibroin proteins) and the degree to which they depend on this ability. There are over 34,000 described species of Araneae (*1*). Each species utilizes silk, and some ecribellate orb-weavers (Araneoidea) have a varied toolkit of task-specific silks with divergent mechanical properties (*2*). Araneoid major ampullate silk, the primary dragline, is extremely tough. Minor ampullate silk, used in web construction, has high tensile strength. An orb-web's capture spiral, in part composed of flagelliform silk, is elastic and can triple in length before breaking (*3*). Each of these fibers is composed of one or more proteins encoded by the spider silk fibroin gene family (*4*). Sequencing of araneoid fibroins has revealed that these fibroins are dominated by iterations of four simple amino acid motifs: poly-alanine (A_n), alternating glycine and alanine (GA), GGX (where X represents a small subset of amino acids), and $GPG(X)_n$ (*5*).

Spiders draw fibers from dissolved fibroin proteins that are stored in specialized sets of abdominal glands. Each type of silk is secreted and stored by a different abdominal gland until extruded by tiny spigots on the spinnerets. Spiders use fibroin proteins singly or in combination for a variety of different purposes, including: draglines, retreats, egg sacs, and prey-catching snares. Given these specialized applications, individual silks appear to have evolved to possess mechanical properties (e.g., tensile strength and flexibility) that optimize their utility for particular applications.

Orb web spiders like *Nephila* are known to produce spider silk proteins derived from several types of silk

synthetic glands and are designated according to their organ of origin. Spider silk proteins known to exist include: major ampullate spider proteins (MaSp), minor ampullate spider proteins (MiSp), and flagelliform (Flag), tubuliform, aggregate, aciniform, and pyriform spider silk proteins. Spider silk proteins derived from each organ are generally distinguishable from those derived from other synthetic organs by virtue of their physical and chemical properties, which render them well suited to different uses. Tubuliform silk, for example, is used in the outer layers of egg-sacs, whereas aciniform silk is involved in wrapping prey and pyriform silk is laid down as the attachment disk.

Most molecular and structural investigations of spider silks have focused on dragline silk, which has an extraordinarily high tensile strength (e.g. Xu & Lewis, Proc. Natl. Acad. Sci., USA 87, 7120-7124, 1990; Hinman & Lewis, J. Biol. Chem. 267, 19320-19324, 1992; Thiel et al., Biopolymers 34, 1089-1097, 1994; Simmons et al., Science 271, 84-87, 1996; Kümmerlen et al., Macromol. 29, 2920-2928, 1996; and Osaki, Nature 384, 419, 1996). Dragline silk, often referred to as major ampullate silk because it is produced by the major ampullate glands, has a high tensile strength (5×10^9 Nm^{-2}) similar to Kevlar (4×10^9 Nm^{-2}) (Gosline et al., Endeavour 10, 37-43, 1986; Stauffer et al., J. Arachnol. 22, 5-11, 1994). In addition to this exceptional strength, dragline silk also exhibits substantial (~35%) elasticity (Gosline et al., Endeavour 10, 37-43, 1986). Thus a structure/function analysis of dragline silk is revealing in terms of the features of a protein which confer strength and elasticity.

Silk strength is widely attributed to crystalline beta-sheet structures. Such protein domains are found in both lepidopteran silks (e.g. Bombyx mori, Mita et al.,

J. Mol. Evol. 38, 583-592, 1994) and spider silks (Xu & Lewis, Proc. Natl. Acad. Sci., USA 87, 7120-7124, 1990; Hinman & Lewis, J. Biol. Chem. 267, 19320-19324, 1992; Gosline et al., Endeavour 10, 37-43, 1986). In contrast, elasticity is generally thought to involve amorphous regions (Wainwright et al., Mechanical design in organisms, Princeton University Press, Princeton, 1982). More precise characterization of these amorphous components can be revealed by molecular sequence data.

Based on the protein sequences of major ampullate silk proteins, a beta-turn structure was suggested to be the likely mechanism of elasticity (Hinman & Lewis, J. Biol. Chem. 267, 19320-19324, 1992). Assessing this proposition, however, was problematic because dragline silk is a hybrid of at least two distinct proteins which impart both strength and moderate elasticity.

Nephila minor ampullate silk can be distinguished from *Nephila* major ampullate silk by both physical and chemical properties. On a basic level, the amino acid composition of solubilized minor ampullate silk differs from that of solubilized major ampullate silk. Like the major ampullate silk proteins (major spidroin 1, MaSP1; major spidroin 2, MaSP2), the proteins comprising minor ampullate silk (minor spindroin 1, MiSP1; minor spindroin 2, MiSP2) have a primary structure dominated by imperfect repetition of a short sequence of amino acids. Moreover, in contrast to the elasticity exhibited by major ampullate silk, minor ampullate silk yields without recoil. Minor ampullate silk will stretch to about 25% of its initial length before breaking, thereby exhibiting a tensile strength of nearly 100,000 pounds per square inch (psi). The minor ampullate silk proteins, therefore, exhibit comparatively lower tensile strength and elasticity relative to major ampullate silk proteins.

The capture spiral, on the other hand, is formed

from silk proteins derived from the flagelliform and aggregate silk glands. The capture spiral of an orb-web comprises a structure having significant ability to stretch, as would be anticipated for a structure that must capture and retain prey. The capture thread has a lower tensile strength (1 X 10^9 Nm^{-2}) but several times the elasticity (>200%) of dragline silk (Vollrath & Edmonds, Nature 340, 305-307, 1989; Kohler & Vollrath, J. Exp. Zool. 271, 1-17, 1995). The flagelliform silk comprises the core fiber of the spiral, while aggregate silk provides a non-fibrous, aqueous coating. Thus, while aggregate silk is an integral part of the elastic capture spiral, it is flagelliform silk that provides the ability to stretch.

SUMMARY OF THE INVENTION

In view of the unique properties of different silks produced by spiders, the identification of novel spider silk proteins and characterization of their chemical and physical properties provide useful new reagents having utility for a number of applications. Spider silk proteins are unique in that they possess properties which include, but are not limited to, high tensile strength and elasticity. Moreover, individual spider silk proteins have evolved to possess different combinations of properties that contribute to the physical balance between protein strength and elasticity.

Spider silk is composed of fibers formed from proteins. Naturally occurring spider silk fibers can be composites of two or more proteins. In general, spider silk proteins are found to have primary amino acid sequences that can be characterized as indirect repeats of a short consensus sequence. Variation in the consensus sequence is then responsible for the distinguishable properties of different silk proteins.

Silk fibers can be made from synthetic polypeptides having amino acid sequences substantially similar to a consensus repeat unit of a silk protein or from polypeptides expressed from nucleic acid sequences encoding a natural or engineered silk protein, or derivative thereof. Depending on the application for which a synthetic spider silk protein is intended, it may also be desirable to form fibers from a single spider silk protein or combinations of different spider silk proteins, the ratio of which can be modified accordingly.

According to one aspect of the invention, nucleic acid sequences encoding novel spider silk proteins are provided. Exemplary nucleic acid sequences of the invention have sequences comprising SEQ ID NOS: 1-28.

In a particular aspect of the invention, exemplary nucleic acid sequences encoding novel MaSp1-like spider silk proteins are provided. Exemplary nucleic acid sequences of this type have sequences comprising SEQ ID NOs: 1-7.

In another aspect of the invention, exemplary nucleic acid sequences encoding novel MaSp2-like spider silk proteins are provided. Exemplary nucleic acid sequences of this type have sequences comprising SEQ ID NOs: 8-16.

In another aspect of the invention, exemplary nucleic acid sequences encoding novel flagelliform (flag)-like spider silk proteins are provided. Exemplary nucleic acid sequences of this type have sequences comprising SEQ ID NOs: 17 and 18.

In another aspect of the invention, nucleic acid sequences encoding novel spider silk proteins are provided. Exemplary nucleic acid sequences of this type have sequences comprising SEQ ID NOs: 19 and 20.

In yet another aspect of the invention, nucleic acid sequences encoding novel spider silk proteins which

comprise atypical repetitive motifs are provided. Exemplary nucleic acid sequences of this type have sequences comprising SEQ ID NOs: 21-27.

In a particular aspect of the invention, an isolated nucleic acid sequence which encodes a novel spider silk protein comprising atypical repetitive motifs is provided. An exemplary nucleic acid sequence of this type has a sequence comprising SEQ ID NO: 28.

In a preferred embodiment of the invention, the isolated nucleic acid molecules provided encode spider silk proteins. In a particularly preferred embodiment, spider silk proteins of the present invention have amino acid sequences comprising SEQ ID NOS: 29-56.

In a particular aspect of the invention, novel MaSp1-like spider silk proteins have amino acid sequences comprising SEQ ID NOs: 29-35.

In another aspect of the invention, novel MaSp2-like spider silk proteins have amino acid sequences comprising SEQ ID NOs: 36-44.

In another aspect of the invention, novel flag-like spider silk proteins have amino acid sequences comprising SEQ ID NOs: 45 and 46.

In another aspect of the invention, novel spider silk proteins have amino acid sequences comprising SEQ ID NOs: 47 and 48.

In yet another aspect of the invention, novel spider silk proteins comprising atypical repetitive motifs have amino acid sequences comprising SEQ ID NOs: 49-55.

In a particular aspect of the invention, a novel spider silk protein comprising atypical repetitive motifs is provided. An exemplary spider silk protein amino acid sequence of this type comprises SEQ ID NO: 56.

According to another aspect of the present invention, an isolated nucleic acid molecule is provided, which has a sequence selected from the group consisting

of: (1) SEQ ID NOs: 1-28; (2) a sequence specifically hybridizing with preselected portions or all of an individual complementary strand of SEQ ID NOs: 1-28 comprising nucleic acids encoding amino acids of SEQ ID NOs: 29-56; (3) a sequence encoding preselected portions of SEQ ID NOs: 1-28, and (4) a sequence comprising nucleic acids encoding amino acids of a consensus sequence (SEQ ID NO: 57) which was derived from SEQ ID NO: 56.

Such partial sequences are useful as probes to identify and isolate homologues of spider silk protein genes of the invention. Additionally, isolated nucleic acid sequences encoding natural allelic variants of the nucleic acids of SEQ ID NOs: 1-28 are also contemplated to be within the scope of the present invention. The term natural allelic variants will be defined hereinbelow.

According to another aspect of the present invention, antibodies immunologically specific for the spider silk proteins described hereinabove are provided.

In yet another aspect of the invention, host cells comprising at least one of the spider silk protein encoding nucleic acids are provided. Such host cells include but are not limited to bacterial cells, fungal cells, insect cells, mammalian cells, and plant cells. Host cells overexpressing one or more of the spider silk protein encoding nucleic acids of the invention provide valuable reagents for many applications, including, but not limited to, production of silk fibers comprising at least one silk protein that can be incorporated into a material to modulate the structural properties of the material.

Naturally occurring spider silk proteins have an imperfectly repetitive structure. Imperfections in the repetition are likely to be a consequence of the process

by which the silk protein genes evolved, rather than a requirement for fiber formation. Imperfections in repetition are thus not likely to affect properties of fibers formed following aggregation of protein molecules.

Accordingly, in another embodiment of the present invention nucleic acid sequences are provided which encode engineered spider silk proteins, each of which comprises a polypeptide having direct repeats of a unit amino acid sequence. Alternatively, nucleic acid sequences may include several different unit amino acid sequences to form a "copolymer" silk protein.

In yet another embodiment of the present invention a spider silk protein expressed from a nucleic acid sequence is provided, wherein the nucleic acid sequence is obtained from cDNA, genomic DNA, synthetic DNA, or fragments of all of the above, derived from a spider ampullate gland.

In another embodiment of the present invention fibers made from silk protein obtained by expression of nucleic acid sequences encoding at least one spider silk protein are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an analysis of phylogenetic relationships of Araneae based on morphological evidence (*1,27*). Previously published spider fibroin sequences are from the two genera marked by white circles. Including data presented herein, fibroin sequences have been characterized for the taxa in red. Circles at internal nodes mark fossil calibration points. Extinct taxa that calibrate these nodes, *Macryphantes* (*16* - black circle) and *Rosamygale* (*13* - gray circle), are indicated, and higher level taxa are to the right of brackets. *Dolomedes*, *Plectreurys*, and *Euagrus* are from the families

Pisauridae, Plectreuridae, and Dipluridae, respectively.

Figure 2 shows consensus ensemble repeat units for non-araneoid spider fibroins. Single letter symbols for amino acids are used, and GGX, GA, and A_n motifs are indicated in green, brown, and red, respectively. *Plectreurys* cDNA1 and *Plectreurys* cDNA2 were derived from the larger ampule-shaped glands of *Plectreurys*, and *Plectreurys* cDNA3 and *Plectreurys* cDNA4 were from the smaller ampullate glands of this spider.

Figure 3 shows consensus ensemble repeat units for four araneoid fibroin orthologue groups. Single letter symbols for amino acids are used, and GGX, GA, A_n, and $GPG(X)_n$ motifs are indicated in green, brown, red, and blue, respectively. The "[spacer]" region of the MiSp fibroins is a serine-rich sequence that is 137 amino acids long in *Nephila clavipes* (Genbank #AF027735). *Nep.c.=Nephila clavipes*, *Nep.m.=N. madagascariensis*, *Nep.s.=N. senegalensis*, *Tet.k.=Tetragnatha kauaiensis*, *Tet.v.=T. versicolor*, *Lat.g.=Latrodectus geometricus*, *Arg.t.=Argiope trifasciata*, *Arg.a.=A. aurantia*, *Ara.b.=Araneus bicentenarius*, *Ara.d.=A. diadematus*, *Gas.m.=Gasteracantha mammosa*. §m=cDNA from major ampullate glands, §f=cDNA from flagelliform glands, †=PCR/genomic clone, *=previously published sequence. The previous designations for *A. diadematus* fibroins (*4*) are shown in parentheses (ADF1-4).

DETAILED DESCRIPTION OF THE INVENTION

The physical characteristics of spider silk proteins confer unparalleled mechanical properties to these fibroins and, thus, render spider silk proteins ideally suited to a variety of applications. Identification of

novel spider silk proteins as described herein, therefore, provides useful tools for the generation of natural and synthetic spider silk proteins which can be woven into fibers to imbue fibers comprised of such proteins with unique properties.

In a preferred embodiment of the invention, nucleic acid sequences encoding novel spider silk proteins have sequences comprising SEQ ID NOS: 1-28.

In a particularly preferred embodiment, spider silk proteins of the present invention have amino acid sequences comprising SEQ ID NOS: 29-56.

In yet another preferred embodiment, a consensus sequence derived from SEQ ID NO: 56 has amino acid sequences comprising SEQ ID NO: 57.

Other spider silk proteins have been previously identified, see for example U.S. patent application Nos. 5,773,771; 5,989,894; and 5,728,810, the entire disclosures of which are incorporated herein by reference.

I. Definitions

The following definitions are provided to facilitate an understanding of the present invention:

With reference to nucleic acids used in the invention, the term "isolated nucleic acid" is sometimes employed. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it was derived. For example, the "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. An "isolated nucleic acid molecule" may also comprise a cDNA molecule. An isolated

nucleic acid molecule inserted into a vector is also sometimes referred to herein as a recombinant nucleic acid molecule.

With respect to RNA molecules, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form.

With respect to single stranded nucleic acids, particularly oligonucleotides, the term "specifically hybridizing" refers to the association between two single-stranded nucleotide molecules of sufficiently complementary sequence to permit such hybridization under pre-determined conditions generally used in the art (sometimes termed "substantially complementary"). In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-stranded DNA or RNA molecule of the invention, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence. Appropriate conditions enabling specific hybridization of single stranded nucleic acid molecules of varying complementarity are well known in the art.

For instance, one common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is set forth below (Sambrook et al., 1989):

$T_m = 81.5°C + 16.6\text{Log}\,[Na+] + 0.41(\%\ G+C) - 0.63\ (\%\ \text{formamide}) - 600/\#bp\ \text{in duplex}$

As an illustration of the above formula, using [Na+] = [0.368] and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C.

The term "oligonucleotide," as used herein refers to primers and probes of the present invention, and is defined as a nucleic acid molecule comprised of two or more ribo- or deoxyribonucleotides, preferably more than three. The exact size of the oligonucleotide will depend on various factors and on the particular application and use of the oligonucleotide. Preferred oligonucleotides comprise 15-50 consecutive bases of SEQ ID Nos: 1-28.

The term "probe" as used herein refers to an oligonucleotide, polynucleotide or nucleic acid, either RNA or DNA, whether occurring naturally as in a purified restriction enzyme digest or produced synthetically, which is capable of annealing with or specifically hybridizing to a nucleic acid with sequences complementary to the probe. A probe may be either single-stranded or double-stranded. The exact length of the probe will depend upon many factors, including temperature, source of probe and use of the method. For example, depending on the complexity of the target sequence, the oligonucleotide probe typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. The probes herein are selected to be complementary to different strands of a particular target nucleic acid sequence. This means that the probes must be sufficiently complementary so as to be able to "specifically hybridize" or anneal with their respective target strands under a set of pre-determined conditions. Therefore, the probe sequence need not reflect the exact

complementary sequence of the target. For example, a non-complementary nucleotide fragment may be attached to the 5' or 3' end of the probe, with the remainder of the probe sequence being complementary to the target strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the sequence of the target nucleic acid to anneal therewith specifically.

The term "primer" as used herein refers to an oligonucleotide, either RNA or DNA, either single-stranded or double-stranded, either derived from a biological system, generated by restriction enzyme digestion, or produced synthetically which, when placed in the proper environment, is able to functionally act as an initiator of template-dependent nucleic acid synthesis. When presented with an appropriate nucleic acid template, suitable nucleoside triphosphate precursors of nucleic acids, a polymerase enzyme, suitable cofactors and conditions such as a suitable temperature and pH, the primer may be extended at its 3' terminus by the addition of nucleotides by the action of a polymerase or similar activity to yield a primer extension product. The primer may vary in length depending on the particular conditions and requirement of the application. For example, in diagnostic applications, the oligonucleotide primer is typically 15-25 or more nucleotides in length. The primer must be of sufficient complementarity to the desired template to prime the synthesis of the desired extension product, that is, to be able to anneal with the desired template strand in a manner sufficient to provide the 3' hydroxyl moiety of the primer in appropriate juxtaposition for use in the initiation of synthesis by a polymerase or similar enzyme. It is not required that the primer sequence

represent an exact complement of the desired template. For example, a non-complementary nucleotide sequence may be attached to the 5' end of an otherwise complementary primer. Alternatively, non-complementary bases may be interspersed within the oligonucleotide primer sequence, provided that the primer sequence has sufficient complementarity with the sequence of the desired template strand to functionally provide a template-primer complex for the synthesis of the extension product.

Polymerase chain reaction (PCR) has been described in US Patents 4,683,195, 4,800,195, and 4,965,188, the entire disclosures of which are incorporated by reference herein.

Amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form may be substituted for any L-amino acid residue, provided the desired properties of the polypeptide are retained. All amino-acid residue sequences represented herein conform to the conventional left-to-right amino-terminus to carboxy-terminus orientation.

Amino acid residues are identified in the present application according to the three-letter or one-letter abbreviations in the following Table:

TABLE 1

Amino Acid	3-letter Abbreviation	1-letter Abbreviation
L-Alanine	Ala	A
L-Arginine	Arg	R
L-Asparagine	Asn	N
L-Aspartic Acid	Asp	D
L-Cysteine	Cys	C
L-Glutamine	Gln	Q
L-Glutamic Acid	Glu	E
Glycine	Gly	G
L-Histidine	His	H
L-Isoleucine	Ile	I
L-Leucine	Leu	L
L-Methionine	Met	M
L-Phenylalanine	Phe	F
L-Proline	Pro	P
L-Serine	Ser	S
L-Threonine	Thr	T
L-Tryptophan	Trp	W
L-Tyrosine	Tyr	Y
L-Valine	Val	V
L-Lysine	Lys	K

The term "isolated protein" or "isolated and purified protein" is sometimes used herein. This term refers primarily to a protein produced by expression of an isolated nucleic acid molecule of the invention. Alternatively, this term may refer to a protein that has been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in

"substantially pure" form. "Isolated" is not meant to exclude artificial or synthetic mixtures with other compounds or materials, or the presence of impurities that do not interfere with the fundamental activity, and that may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into, for example, immunogenic preparations or pharmaceutically acceptable preparations.

"Mature protein" or "mature polypeptide" shall mean a polypeptide possessing the sequence of the polypeptide after any processing events that normally occur to the polypeptide during the course of its genesis, such as proteolytic processing from a polyprotein precursor. In designating the sequence or boundaries of a mature protein, the first amino acid of the mature protein sequence is designated as amino acid residue 1. As used herein, any amino acid residues associated with a mature protein not naturally found associated with that protein that precedes amino acid 1 are designated amino acid -1, -2, -3 and so on. For recombinant expression systems, a methionine initiator codon is often utilized for purposes of efficient translation. This methionine residue in the resulting polypeptide, as used herein, would be positioned at -1 relative to the mature protein sequence.

A low molecular weight "peptide analog" shall mean a natural or mutant (mutated) analog of a protein, comprising a linear or discontinuous series of fragments of that protein and which may have one or more amino acids replaced with other amino acids and which has altered, enhanced or diminished biological activity when compared with the parent or nonmutated protein.

The term "biological activity" is a function or set of functions performed by a molecule in a biological context (i.e., in an organism or an in vitro surrogate or facsimile model). For spider silk proteins, biological

activity is characterized by physical properties (e.g., tensile strength and elasticity) as described herein.

The term "substantially pure" refers to a preparation comprising at least 50-60% by weight the compound of interest (e.g., nucleic acid, oligonucleotide, polypeptide, protein, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest. Purity is measured by methods appropriate for the compound of interest (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, mass spectrometry and the like).

The term "tag," "tag sequence" or "protein tag" refers to a chemical moiety, either a nucleotide, oligonucleotide, polynucleotide or an amino acid, peptide or protein or other chemical, that when added to another sequence, provides additional utility or confers useful properties, particularly in the detection or isolation, of that sequence. Thus, for example, a homopolymer nucleic acid sequence or a nucleic acid sequence complementary to a capture oligonucleotide may be added to a primer or probe sequence to facilitate the subsequent isolation of an extension product or hybridized product. In the case of protein tags, histidine residues (e.g., 4 to 8 consecutive histidine residues) may be added to either the amino- or carboxy-terminus of a protein to facilitate protein isolation by chelating metal chromatography. Alternatively, amino acid sequences, peptides, proteins or fusion partners representing epitopes or binding determinants reactive with specific antibody molecules or other molecules (e.g., flag epitope, c-myc epitope, transmembrane epitope of the influenza A virus hemaglutinin protein, protein A, cellulose binding

domain, calmodulin binding protein, maltose binding protein, chitin binding domain, glutathione S-transferase, and the like) may be added to proteins to facilitate protein isolation by procedures such as affinity or immunoaffinity chromatography. Chemical tag moieties include such molecules as biotin, which may be added to either nucleic acids or proteins, and facilitates isolation or detection by interaction with avidin reagents, and the like. Numerous other tag moieties are known to, and can be envisioned by the trained artisan, and are contemplated to be within the scope of this definition.

A "vector" is a replicon, such as a plasmid, cosmid, bacmid, phage or virus, to which another genetic sequence or element (either DNA or RNA) may be attached so as to bring about the replication of the attached sequence or element. An "expression vector" is a specialized vector that contains a gene with the necessary regulatory regions needed for expression in a host cell.

The term "operably linked" means that the regulatory sequences necessary for expression of the coding sequence are placed in the DNA molecule in the appropriate positions relative to the coding sequence so as to effect expression of the coding sequence. This same definition is sometimes applied to the arrangement of coding sequences and transcription control elements (e.g. promoters, enhancers, and termination elements) in an expression vector. This definition is also sometimes applied to the arrangement of nucleic acid sequences of a first and a second nucleic acid molecule wherein a hybrid nucleic acid molecule is generated.

The phrase "consisting essentially of" when referring to a particular nucleotide or amino acid means a sequence having the properties of a given SEQ ID NO:. For example, when used in reference to an amino acid

sequence, the phrase includes the sequence per se and molecular modifications that would not affect the basic and novel characteristics of the sequence.

A "clone" or "clonal cell population" is a population of cells derived from a single cell or common ancestor by mitosis.

A "cell line" is a clone of a primary cell or cell population that is capable of stable growth *in vitro* for many generations.

An "immune response" signifies any reaction produced by an antigen, such as a viral antigen, in a host having a functioning immune system. Immune responses may be either humoral in nature, that is, involve production of immunoglobulins or antibodies, or cellular in nature, involving various types of B and T lymphocytes, dendritic cells, macrophages, antigen presenting cells and the like, or both. Immune responses may also involve the production or elaboration of various effector molecules such as cytokines, lymphokines and the like. Immune responses may be measured both in *in vitro* and in various cellular or animal systems. Such immune responses may be important in protecting the host from disease and may be used prophylactically and therapeutically.

An "antibody" or "antibody molecule" is any immunoglobulin, including antibodies and fragments thereof, that binds to a specific antigen. The term includes polyclonal, monoclonal, chimeric, and bispecific antibodies. As used herein, antibody or antibody molecule contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule such as those portions known in the art as Fab, Fab', F(ab')2 and F(v).

With respect to antibodies, the term "immunologically specific" refers to antibodies that bind to one or more epitopes of a protein or compound of

interest, but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

"Natural allelic variants", "mutants" and "derivatives" of particular sequences of nucleic acids refer to nucleic acid sequences that are closely related to a particular sequence but which may possess, either naturally or by design, changes in sequence or structure. By closely related, it is meant that at least about 75%, but often, more than 90%, of the nucleotides of the sequence match over the defined length of the nucleic acid sequence referred to using a specific SEQ ID NO. Changes or differences in nucleotide sequence between closely related nucleic acid sequences may represent nucleotide changes in the sequence that arise during the course of normal replication or duplication in nature of the particular nucleic acid sequence. Other changes may be specifically designed and introduced into the sequence for specific purposes, such as to change an amino acid codon or sequence in a regulatory region of the nucleic acid. Such specific changes may be made *in vitro* using a variety of mutagenesis techniques or produced in a host organism placed under particular selection conditions that induce or select for the changes. Such sequence variants generated specifically may be referred to as "mutants" or "derivatives" of the original sequence.

A "derivative" of a spider silk protein or a fragment thereof means a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve insertion, addition, deletion or substitution of one or more amino acids, and may or may not alter the essential activity of original the spider silk protein.

As mentioned above, the spider silk polypeptide or protein of the invention includes any analogue, fragment, derivative or mutant which is derived from a spider silk protein and which retains at least one property or other characteristic of a spider silk protein. Different "variants" of spider silk proteins exist in nature. These variants may be alleles characterized by differences in the nucleotide sequences of the gene coding for the protein, or may involve different RNA processing or post-translational modifications. The skilled person can produce variants having single or multiple amino acid substitutions, deletions, additions or replacements. These variants may include inter alia: (a) variants in which one or more amino acids residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to a spider silk protein, (c) variants in which one or more amino acids include a substituent group, and (d) variants in which a spider silk protein or fragment thereof is fused with another peptide or polypeptide such as a fusion partner, a protein tag or other chemical moiety, that may confer useful properties to a spider silk protein, such as, for example, an epitope for an antibody, a polyhistidine sequence, a biotin moiety and the like. Other spider silk proteins of the invention include variants in which amino acid residues from one species are substituted for the corresponding residue in another species, either at the conserved or non-conserved positions. In another embodiment, amino acid residues at non-conserved positions are substituted with conservative or non-conservative residues. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic techniques are known to the person having ordinary skill

in the art.

To the extent such allelic variations, analogues, fragments, derivatives, mutants, and modifications, including alternative nucleic acid processing forms and alternative post-translational modification forms result in derivatives of spider silk protein that retain any of the biological properties of a spider silk protein, they are included within the scope of this invention.

The term "functional" as used herein implies that the nucleic or amino acid sequence is functional for the recited assay or purpose.

A "unit repeat" constitutes a repetitive short sequence. Thus, the primary structure of the spider silk proteins is considered to consist mostly of a series of small variations of a unit repeat. The unit repeats in the naturally occurring proteins are often distinct from each other. That is, there is little or no exact duplication of the unit repeats along the length of the protein. Synthetic spider silks, however, can be made wherein the primary structure of the protein comprises a number of exact repetitions of a single unit repeat. Additional synthetic spider silks can be synthesized which comprise a number of repetitions of one unit repeat together with a number of repetitions of a second unit repeat. Such a structure would be similar to a typical block copolymer. Unit repeats of several different sequences can also be combined to provide a synthetic spider silk protein having properties suited to a particular application.

The term "direct repeat" as used herein is a repeat in tandem (head-to-tail arrangement) with a similar repeat.

II. Preparation of Spider Silk-Encoding Nucleic Acid Molecules, Spider Silk Proteins, and Antibodies Thereto

A. Nucleic Acid Molecules

Nucleic acid molecules encoding the polypeptides of the invention may be prepared by two general methods: (1) synthesis from appropriate nucleotide triphosphates, or (2) isolation from biological sources. Both methods utilize protocols well known in the art. The availability of nucleotide sequence information, such as the DNA sequences encoding a spider silk protein, enables preparation of an isolated nucleic acid molecule of the invention by oligonucleotide synthesis. Synthetic oligonucleotides may be prepared by the phosphoramidite method employed in the Applied Biosystems 38A DNA Synthesizer or similar devices. The resultant construct may be used directly or purified according to methods known in the art, such as high performance liquid chromatography (HPLC).

Specific probes for identifying such sequences as a spider silk protein encoding sequence may be between 15 and 40 nucleotides in length. For probes longer than those described above, the additional contiguous nucleotides are provided within sequences encoding a spider silk protein.

In accordance with the present invention, nucleic acids having the appropriate level of sequence homology with sequences encoding a spider silk protein may be identified by using hybridization and washing conditions of appropriate stringency. For example, hybridizations may be performed, according to the method of Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory (1989), using a hybridization solution comprising: 5X SSC, 5X Denhardt's reagent, 1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is

carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2 X SSC and 1% SDS; (2) 15 minutes at room temperature in 2 X SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37°C in 1 X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1 X SSC and 1% SDS, changing the solution every 30 minutes.

The nucleic acid molecules described herein include cDNA, genomic DNA, RNA, and fragments thereof which may be single- or double-stranded. Thus, oligonucleotides are provided having sequences capable of hybridizing with at least one sequence of a nucleic acid sequence, such as selected segments of sequences encoding a spider silk protein. Also contemplated in the scope of the present invention are methods of use for oligonucleotide probes which specifically hybridize with DNA from sequences encoding a spider silk protein under high stringency conditions. Primers capable of specifically amplifying sequences encoding a spider silk protein are also provided. As mentioned previously, such oligonucleotides are useful as primers for detecting, isolating and amplifying sequences encoding a spider silk protein.

Antisense nucleic acid molecules which may be targeted to translation initiation sites and/or splice sites to inhibit the expression of spider silk protein genes or production of their encoded proteins are also provided. Such antisense molecules are typically between 15 and 30 nucleotides in length and often span the translational start site of a spider silk protein mRNA molecule.

B. Proteins

Full-length spider silk proteins of the present invention may be prepared in a variety of ways, according to known methods. The proteins may be purified from

appropriate sources, e.g., transformed bacterial or animal cultured cells or tissues, by immunoaffinity purification. However, this is not a preferred method due to the low levels of protein likely to be present in a given cell type at any time. The availability of nucleic acid molecules encoding spider silk proteins enables production of the proteins using *in vitro* expression methods known in the art. For example, a cDNA or gene may be cloned into an appropriate *in vitro* transcription vector, such as pSP64 or pSP65 for *in vitro* transcription, followed by cell-free translation in a suitable cell-free translation system, such as wheat germ or rabbit reticulocytes. *In vitro* transcription and translation systems are commercially available, e.g., from Promega Biotech, Madison, Wisconsin or Gibco-BRL, Gaithersburg, Maryland.

Alternatively, according to a preferred embodiment, larger quantities of spider silk protein may be produced by expression in a suitable prokaryotic or eukaryotic system. For example, part or all of at least one DNA molecule, such as nucleic acid sequences having a sequence selected from the group of SEQ ID NOs: 1-28 may be inserted into a plasmid vector adapted for expression in a bacterial cell, such as *E. coli*. Such vectors comprise the regulatory elements necessary for expression of the DNA in the host cell positioned in such a manner as to permit expression of the DNA in the host cell. Such regulatory elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The spider silk proteins produced by gene expression in a recombinant prokaryotic or eukaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the

recombinant protein is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein from cell lysates (remains of cells following disruption of cellular integrity) derived from prokaryotic or eukaryotic cells in which a protein was expressed. Methods for generation of such cell lysates are known to those of skill in the art. Recombinant protein can be purified by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant protein or nickel columns for isolation of recombinant proteins tagged with 6-8 histidine residues at their N-terminus or C-terminus. Alternative tags may comprise the FLAG epitope or the hemagglutinin epitope. Such methods are commonly used by skilled practitioners.

The spider silk proteins of the invention, prepared by the aforementioned methods, may be analyzed according to standard procedures. For example, such proteins may be subjected to amino acid sequence analysis, according to known methods.

A protein produced according to the present invention can be chemically modified after synthesis of the polypeptide. The presence of several carboxylic acid side chains (Asp or Glu) in the spacer regions facilitates the attachment of a variety of different chemical groups to silk proteins including amino acids having such side chains. The simplest and easiest procedure is to use a water-soluble carbo-diimide to attach the modifying group via a primary amine. If the group to be attached has no primary amine, a variety of linking agents can be attached via their own primary amines and then the modifying group attached via an available chemistry. Jennes, L. and Stumpf, W. E.

Neuroendocrine Peptide Methodology, Chapter 42. P. Michael Conn, editor. Academic Press, 1989.

Desirable chemical modifications include, but are not limited to, derivatization with peptides that bind to cells, e.g. fibroblasts, derivatization with antibiotics and derivatization with cross-linking agents so that cross-linked fibers can be made. The selection of derivatizing agents for a particular purpose is within the skill of the ordinary practitioner of the art.

Exemplary Methods for Generation of Spider Silk Proteins

In view of the unique properties of spider silk proteins, special considerations should be applied to the generation of synthetic spider silk proteins. The repetitive nature of amino acid sequences encoding these proteins may render synthesis of a full length spider silk protein, or fragments thereof, technically challenging. To facilitate production of full length silk protein molecules, the following protocol is provided.

The polypeptides of the present invention can be made by direct synthesis or by expression from cloned DNA. Means for expressing cloned DNA are set forth above and are generally known in the art. The following considerations are recommended for the design of expression vectors used to express DNA encoding the spider silk proteins of the present invention.

First, since spider silk proteins are highly repetitive in their structure, cloned DNA should be propagated and expressed in host cell strains that can maintain repetitive sequences in extrachromosomal elements (e.g. SURE ™ cells, Stratagene). The prevalence of specific amino acids (e.g., alanine, glycine, proline, and glutamine) also suggests that it might be advantageous to use a host cell that overexpresses tRNA

for these amino acids.

The proteins of the present invention can otherwise be expressed using vectors providing for high level transcription, fusion proteins allowing affinity purification through an epitope tag, and the like. The hosts can be either bacterial or eukaryotic cells. Eukaryotic cells such as yeast, especially *Saccharomyces cerevisisae*, or insect cells might be particularly useful eukaryotic hosts. Expression of an engineered minor ampullate silk protein is described in U.S. Pat. No. 5,756,677, herein incorporated by reference. Such an approach can be used to express proteins of the present invention.

A useful spider silk protein or fragment thereof may be (1) insoluble inside a cell in which it is expressed or (2) capable of being formed into an insoluble fiber under normal conditions by which fibers are made. Preferably, the protein is insoluble under conditions (1) and (2). Specifically, the protein or fragment may be insoluble in a solvent such as water, alcohol (methanol, ethanol, etc.), acetone and/or organic acids, etc. The spider silk protein or fragment thereof should be capable of being formed into a fiber having high tensile strength, e.g., a tensile strength of 0.5x to 2x wherein x is the tensile strength of a fiber formed from a corresponding natural silk or whole protein. A spider silk protein or fragment thereof should also be capable of being formed into a fiber possessing high elasticity, e.g., at least 15%, more preferably about 25%.

Variants of a spider silk protein may be formed into a fiber having a tensile strength and/or elasticity which is greater than that of the natural spider silk or natural protein. The elasticity may be increased up to 100%. Variants may also possess properties of protein fragments.

A fragment or variant may have substantially the same characteristics as a natural spider silk. The natural protein may be particularly insoluble when in fiber form and resistant to degradation by most enzymes.

Recombinant spider silk proteins may be recovered from cultures by lysing cells to release spider silk proteins expressed therein. Initially, cell debris can be separated by centrifugation. Clarified cell lysate comprised of debris and supernatant can then be repeatedly extracted with solvents in which spider silk proteins are insoluble, but cellular debris is soluble. A differential solubilization process such as described above can be used to facilitate isolation of a purified spider silk protein precipitate. These procedures can be repeated and combined with other procedures including filtration, dialysis and/or chromatography to obtain a pure product.

Fibrillar aggregates will form from solutions by spontaneous self-assembly of spider silk proteins when the protein concentration exceeds a critical value. The aggregates can be gathered and mechanically spun into macroscopic fibers according to the method of O'Brien et al. [I. O'Brien et al., "Design, Synthesis and Fabrication of Novel Self-Assembling Fibrillar Proteins", in Silk Polymers: Materials Science and Biotechnology, pp. 104-117, Kaplan, Adams, Farmer and Viney, eds., c. 1994 by American Chemical Society, Washington, D.C.].

Exemplary Methods for Preparation of Fibers From Spider Silk Proteins

As noted above, the spider silk proteins can be viewed as derivatized polyamides. Accordingly, methods for producing fiber from soluble spider silk proteins are similar to those used to produce typical polyamide fibers, e.g. nylons, and the like.

O'Brien et al. *supra* describe fiber production from adenovirus fiber proteins. In a typical fiber production, spider silk proteins can be solubilized in a strongly polar solvent. The protein concentration of such a protein solution should typically be greater than 5% and is preferably between 8 and 20%.

Fibers should preferably be spun from solutions having properties characteristic of a liquid crystal phase. The fiber concentration at which phase transition can occur is dependent on the polypeptide composition of a protein or combination of proteins present in the solution. Phase transition, however, can be detected by monitoring the clarity and birefringence of the solution. Onset of a liquid crystal phase can be detected when the solution acquires a translucent appearance and registers birefringence when viewed through crossed polarizing filters.

The solvent used to dissolve a spider silk protein should be polar, and is preferably highly polar. Such solvents are exemplified by di- and tri- haloacetic acids, and haloalcohols (e.g. hexafluoroisopropanol). In some instances, co-solvents such as acetone are useful. Solutions of chaotropic agents, such as lithium thiocyanate, guanidine thiocyanate or urea can also be used.

In one fiber-forming technique, fibers can first be extruded from the protein solution through an orifice into methanol, until a length sufficient to be picked up by a mechanical means is produced. Then a fiber can be pulled by such mechanical means through a methanol solution, collected, and dried. Methods for drawing fibers are considered well-known in the art. For example, fibers made from a 58 kDa synthetic MaSp consensus polypeptide were drawn by methods similar to those used for drawing low molecular weight nylons. Such

methods are described in U.S. Pat. No. 5,994,099, the entirety of which is incorporated herein by reference.

Of note, spider silk proteins of the present invention have primary structures dominated by imperfect repetition of a short sequence of amino acids. A "unit repeat" constitutes one such short sequence. Thus, the primary structure of a spider silk protein can be thought to consist mostly of a series of small variations of a unit repeat. Unit repeats in a naturally occurring protein are often distinct from each other. In other words, there is little or no exact duplication of a unit repeat along the length of a protein. Synthetic spider silks, however, can be generated wherein the primary structure of a synthetic spider silk protein can be described as a number of exact repetitions of a single unit repeat. Additional synthetic spider silks can be described as a number of repetitions of one unit repeat together with a number of repetitions of a second unit repeat. Such a structure would be similar to a typical block copolymer. The present invention also encompasses generation of synthetic spider silk proteins comprising unit repeats derived from several different spider silk sequences (naturally occurring variants or genetically engineered variants thereof).

Such synthetic hybrid spider silk proteins may each have 900 to 2700 amino acids with 25 to 100, preferably 30 to 90 repeats. A spider silk or fragment or variant thereof usually has a molecular weight of at least about 16,000 daltons, preferably 16,000 to 150,000 daltons, more preferably 50,000 to 120,000 daltons for fragments and greater than 100,000 but less than 500,000 daltons, preferably 120,000 to 350,000 for a full length protein.

C. Antibodies

The present invention also provides antibodies capable of immunospecifically binding to proteins of the invention. Polyclonal antibodies directed toward a spider silk protein may be prepared according to standard methods. In a preferred embodiment, monoclonal antibodies are prepared, which react immunospecifically with various epitopes of the spider silk proteins described herein. Monoclonal antibodies may be prepared according to general methods of Köhler and Milstein, following standard protocols. Polyclonal or monoclonal antibodies that immunospecifically interact with spider silk proteins can be utilized for identifying and purifying such proteins. For example, antibodies may be utilized for affinity separation of proteins with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules. Other uses of anti-spider silk protein antibodies are described below.

III. Uses of Spider Silk-Encoding Nucleic Acids, Spider Silk Proteins and Antibodies Thereto

A. Spider Silk-Encoding Nucleic Acids

Spider silk protein-encoding nucleic acids may be used for a variety of purposes in accordance with the present invention. Spider silk protein-encoding DNA, RNA, or fragments thereof may be used as probes to detect the presence of and/or expression of genes encoding spider silk proteins. Methods in which spider silk protein-encoding nucleic acids may be utilized as probes for such assays include, but are not limited to: (1) *in situ* hybridization; (2) Southern hybridization; (3) northern hybridization; and (4) assorted amplification

reactions such as polymerase chain reactions (PCR).

The spider silk protein-encoding nucleic acids of the invention may also be utilized as probes to identify related genes from other animal species. As is well known in the art, hybridization stringencies may be adjusted to allow hybridization of nucleic acid probes with complementary sequences of varying degrees of homology. Thus, spider silk protein-encoding nucleic acids may be used to advantage to identify and characterize other genes of varying degrees of relation to the spider silk protein genes of the invention. Such information enables further characterization of nucleic acid sequences which encode proteins that possess physical properties typical of spider silk proteins and thus facilitate structure/function analysis of such proteins. Additionally, they may be used to identify genes encoding proteins that interact with spider silk proteins (e.g., by the "interaction trap" technique), which should further accelerate identification of other components utilized in webs comprised of spider silk proteins. Moreover, interacting proteins identified in such screens maybe of utility in the generation and/or optimization of materials comprised of synthetic spider silk proteins. Spider silk protein encoding nucleic acids may also be used to generate primer sets suitable for PCR amplification of target spider silk protein DNA. Criteria for selecting suitable primers are well known to those of ordinary skill in the art.

Host cells comprising at least one spider silk protein encoding DNA molecule are encompassed in the present invention. Host cells contemplated for use in the present invention include but are not limited to bacterial cells, fungal cells, insect cells, mammalian cells, and plant cells. The spider silk protein encoding DNA molecules may be introduced singly into such host

cells or in combination to assess the phenotype of cells conferred by such expression. Methods for introducing DNA molecules are also well known to those of ordinary skill in the art. Such methods are set forth in Ausubel et al. eds., Current Protocols in Molecular Biology, John Wiley & Sons, NY, NY 1995, the disclosure of which is incorporated by reference herein.

As described above, spider silk protein-encoding nucleic acids are also used to advantage to produce large quantities of substantially pure spider silk proteins, or selected portions thereof.

B. Proteins and Antibodies

Purified spider silk protein, or fragments thereof, produced by methods of the present invention can be used to advantage in a variety of different applications, including, but not limited to, production of fabric, sutures, medical coverings, high-tech clothing, rope, reinforced plastics, and other applications in which various combinations of strength and elasticity are required.

Table II lists physical properties of various biological and manmade materials

Material	Material Strength ($N\ m^{-2}$)	Elasticity (%)	Energy to Break ($J\ kg^{-1}$)
Dragline Silk	4×10^9	35	1×10^5
Minor Silk	1×10^9	5	3×10^4
Flagelliform Silk	1×10^9	200+	1×10^5
KEVLAR	4×10^9	5	3×10^4
Rubber	1×10^9	600	8×10^4
Tendon	1×10^9	5	5×10^3

As shown in Table II, spider silks are characterized by advantageous physical properties, including, but not limited to, high tensile strength and pronounced elasticity, that are highly desirable for numerous applications. It is significant to note that spider silks possess these physical properties in aggregation which renders them unique proteins having unparalleled utility. For example, spider dragline silk has a tensile strength greater than steel or carbon fibers (200 ksi), elasticity as great as some nylon (35%), a stiffness as low as silk (0.6 msi), and the ability to supercontract in water (up to 60% decrease in length). In view of its high tensile strength and elasticity, the energy required to break dragline silk exceeds that required to break any known fiber including Kelvar™ and steel. These properties are unmatched by any known natural or manmade material. Moreover, the new materials of the present invention would also provide unique combinations of such desirable features in a very low weight material.

In view of the foregoing advantageous properties, use of the spider silk proteins disclosed in the present invention as components in materials would produce superior products. When spider silk is dissolved in an appropriate solvent and forced through a small orifice to generate spider silk fibers, such fibers can be woven into a fabric/material or added into a composite fabric/material. For example, spider silk fibers can be woven into fabrics to modulate the strength and elasticity of a fabric, thus rendering materials comprising such modified fabric optimized for different applications. Spider silk fibers can be of particular utility when incorporated into materials used to make high-tech clothing, rope, sails, parachutes, wings on aerial devices (e.g., hang gliders), flexible tie downs for electrical components, sutures, and even as a

biomaterial for implantation (e.g., artificial ligaments or aortic banding). Biomedical applications involve use of natural and/or synthetic spider silk fibers of the present invention in sutures used in surgical procedures, including, but not limited to: eye surgery, reconstructive surgery (e.g., nerve or tympanic membrane reconstruction), vascular closure, bowel surgery, cosmetic surgery, and central nervous system surgery. Natural and synthetic spider silk fibers may also be of utility in the generation of antibiotic impregnated sutures and implant material and matrix material for reconstruction of bone and connective tissue. Implants and matrix material for reconstruction may be impregnated with aggregated growth factors, differentiation factors, and/or cell attractants to facilitate incorporation of the exogenous material and optimize recovery of a patient. Spider silk proteins and fibers of the present invention can be used for any application in which various combinations of strength and elasticity are required. Moreover, spider silk proteins can be modified to optimize their utility in any application. As described above, sequences of spider silk proteins can be modified to alter various physical properties of a fibroin and different spider silk proteins and variants thereof can be woven in combination to produce fibers comprised of at least one spider silk protein or variant thereof.

In a preliminary study designed to evaluate the potential for an immune response to a natural spider silk protein, natural dragline silk was implanted into mice and rats intramuscularly, intraperitoneally, or subcutaneously. Animals into which natural dragline silk was introduced did not mount an immune response to the spider silk protein, irrespective of the site of implantation. Of note, tissue sections surrounding

spider silk protein implants were essentially identical to tissue sections derived from implantation sites into which a polyethylene rod was inserted. Since a polyethylene rod was used as the solid matrix about which the dragline spider silk protein was wrapped prior to implantation, introduction of a polyethylene rod alone serves as a negative control for the experiment. In view of the above, spider silk proteins of the present invention are expected to elicit minimal immunological responses when introduced into vertebrate animals.

Synthetic spider silk fibers are of utility in any application for which natural spider silk fibers can be used. For example, synthetic fibers may be mixed with various plastics and/or resins to prepare a fiber-reinforced plastic and/or resin product. Because spider silk is stable up to 180° C., spider silk protein fibers would be of utility as structural reinforcement material in thermal injected plastics.

It should be apparent from the foregoing that the spider silk proteins of the present invention and derivatives thereof can be generated in large quantities by means generally known to those of skill in the art. Spider silk proteins and derivatives thereof can be made into fibers for any intended use. Moreover, mixed composites of fibers are also of interest as a consequence of their unique combined properties. Such mixed composites can confer characteristics of flexibility and strength to any material into which they can be incorporated.

Purified spider silk protein, or fragments thereof, may be used to produce polyclonal or monoclonal antibodies which also may serve as sensitive detection reagents for the presence and accumulation of a spider silk protein (or complexes containing spider silk protein) in cells. Recombinant techniques enable

expression of fusion proteins containing part or all of a spider silk protein. The full length protein or fragments of the protein may be used to advantage to generate an array of monoclonal antibodies specific for various epitopes of a spider silk protein, thereby providing even greater sensitivity for detection of a spider silk protein in cells.

Polyclonal or monoclonal antibodies immunologically specific for a spider silk protein may be used in a variety of assays designed to detect and quantitate these proteins. Such assays include, but are not limited to: (1) flow cytometric analysis; and (2) immunoblot analysis (e.g., dot blot, Western blot) of extracts from various cells. Additionally, anti-spider silk protein antibodies can be used for purification of a spider silk protein and any associated subunits (e.g., affinity column purification, immunoprecipitation).

From the foregoing discussion, it can be seen that spider silk-encoding nucleic acids, spider silk expressing vectors, spider silk and anti-spider silk antibodies of the invention can be used separately or in combination, for example, 1) to identify nucleic acid sequences encoding other spider silk proteins or proteins comprising similar motifs, 2) generate novel hybrid spider silk proteins selected for optimization of different physical properties, 3) to express large quantities of spider silk proteins or fragments or derivatives thereof, and 4) to detect expression of spider silk proteins in cells and/or organisms.

The following examples are provided to illustrate an embodiment of the invention. They are not intended to limit the scope of the invention in any way.

EXAMPLE I

Identification of Clones Encoding Spider Silk Proteins

In order to identify novel spider silk proteins and expand the limited database of nucleic acid sequences encoding fibroin proteins, eleven cDNA libraries derived from silk glands of seven spider genera were generated. cDNA data were supplemented by information from two genomic libraries and PCR-amplified sequences (7). Partial cDNA or gene sequences for 28 fibroins from seven families of Araneae were identified. The data, as described herein, greatly extend the phylogenetic diversity of characterized fibroins (Fig. 1).

Methods for Collection of Sequence Data

cDNA libraries were made from major ampullate glands of *Argiope trifasciata* (Araneidae) and *Lactrodectus geometricus* (Theridiidae), flagelliform glands of *A. trifasciata*, ampullate glands of *Dolomedes tenebrosus* (Pisauridae), two sets of silk glands from *Plectreurys tristis* (Plectreuridae), and silk glands of the mygalomorph *Euagrus chisoseus* (Dipluridae). Glands from *Dolomedes* were the four pairs of spindly ampullate glands with long tails that are connected to the spinnerets via extensive looped ducts. Glands from *Plectreurys* were the two largest pairs of ampule-shaped glands. The relatively uniform silk glands of *Euagrus* were combined in the RNA extraction for this species. Genomic libraries were constructed for *Nephila madagascariensis* (Tetragnathidae) and for *A. trifasciata*. Data from the thirteen libraries were augmented by PCR amplified genomic sequences from eight araneoids.

All the silk glands from *Phidippus audax* (Salticidae) were combined in the RNA extraction for this

species. Similarly, all the silk glands from *Zorocrates* sp. (Zorocratidae) were combined in the RNA extraction for this species. Separate cDNA libraries were made from the aciniform glands connected to the median spinnerets and aciniform glands connected to the posterior spinnerets of *Argiope trifasciata* (Araneidae). Identical cDNA sequences for an aciniform fibroin were isolated from both libraries.

Procedures for construction and screening of the seven cDNA libraries were as follows. Silk glands were dissected from euthanized spiders and flash-frozen in liquid nitrogen. mRNA was extracted from the glands using Dynabeads Oligo(dT)25 (Dynal). cDNA was synthesized using the SuperScript Choice System (Life Technologies) with oligo(dT) as the first-strand synthesis primer. Size-fractionated cDNAs (ChromaSpin-1000, Clontech) were ligated into either pGEM-3zf(+)(Promega) and electroporated into SURE cells (Stratagene), pZErO®-2 cells (Invitrogen) or TOP10 cells (Invitrogen). Eleven libraries of ~1500 recombinant colonies each were constructed, and colonies were replicated onto nylon membranes for screening.

Silk cDNA clones were identified by sequential hybridizations (QuikHyb, Stratagene) with the γ^{32}P-labeled probes CCWAYWCCNCCATATCCWCC (SEQ ID NO: 58), CCWCCWGGWCCNNNWCCWCCWGGWCC (SEQ ID NO: 59), CCWGGWCCTTGTTGWCCWGGWCC (SEQ ID NO: 60), GCDGCDGCDGCDGCDGC (SEQ ID NO: 61), CCWGCWCCWGCWCCWGCWCC (SEQ ID NO: 62), and CCAGADAGACCAGGATTACT (SEQ ID NO: 63) and through the sequencing of clones selected for large insert sizes. The above oligonucleotides were designed based on published spider silk sequences (see 4, 5, 8, 9). Hundreds of positive clones were screened with restriction enzymes, and a subset of clones was sequenced (ABI) using standard M13 sequencing primers and by

inserting transposons with the Genome Priming System (NEB). Divergent transcripts were recognized as members of the spider silk fibroin gene family by internal repetitiveness, sequence similarity to previously sequenced araneoid fibroins in the nonrepetitive COOH-terminus, and consistency of sequence translations with amino acid compositions from dissected silk glands and from published studies (J. Palmer (1985) J. Morphol. 186:195).

Genomic libraries were constructed for the tetragnathid, *Nephila madagascariensis* (λGem-12, Promega), and for the araneid *Argiope trifasciata* (λFixII, Stratagene). The genomic libraries were screened with the radiolabeled probes CCWCCWGGWCCNNNWCCWCCWGGWCC (SEQ ID NO: 59) and CCWGGWCCTTGTTGWCCWGGWCC (SEQ ID NO: 60). Selected silk gene inserts were excised from the λ arms, subcloned into pGEM (Promega) vectors, and sequenced as above.

Genomic sequences were amplified from the araneoids, *N. madagascariensis, N. senegalensis, A. trifasciata, A. aurantia, Tetragnatha kauaiensis, T. versicolor, Latrodectus geometricus,* and *Gasteracantha mammosa*. PCR was by standard procedures (Gibco recombinant Taq polymerase) using primers GGTGCTGGACAAGGAGGATACG (SEQ ID NO: 64), GGCTTGATAAACTGATTGACCAACG (SEQ ID NO: 65), and CACAGCCAGAGAGACCAGGATTGC (SEQ ID NO: 66) for MaSp1 and CCAGGAGGATATGGACCAGGTC (SEQ ID NO: 67), CCGACAACTTGGGCGAACTGAG (SEQ ID NO: 68), CAAGGATCTGGACAGCAAGG (SEQ ID NO: 69), CAACAAGGACCAGGAAGTGGC (SEQ ID NO: 70), CCAACCAWTTGCGCATACTG (SEQ ID NO: 71), GCTTGAGTTAAAGAYTGACC (SEQ ID NO: 72), and GCAGGACCAGGAAGTTATG (SEQ ID NO: 73) for MaSp2. A PCR reaction generally resulted in production of a ladder of DNA fragments. Such ladders result from annealing of PCR

primers to multiple binding sites in the repetitive sequences of a silk gene. For each fibroin amplified, the largest tight band in the PCR ladder was excised and cloned using the TOPO XL PCR cloning kit (Invitrogen). Two to three clones of each silk gene were sequenced as above. Additional sequences from 11 spider fibroins were taken from GenBank (accession numbers M37137, U03848, M92913, AF027735, AFO27736, AFO27737, AF027972, AF027973, AF218623, AF218624, U20328, U47853, U47854, U47855, and U47856). These published sequences are from the araneoid genera, Nephila and Araneus (Fig. 1).

Results

Like previously published fibroins from spiders (*4-5,8-9*) and lepidopterans (*10-11*), the sequences of the invention encode repetitive alanine and glycine-rich proteins. In each molecule, iterated amino acid motifs are organized into higher-order ensemble repeats. Ensemble repeats within each fibroin were aligned, and a consensus ensemble repeat was generated for each molecule (*12*). In part, silk DNA sequences from non-araneoid spiders (Fig. 2) reiterate the importance of amino acid motifs that comprise orb-weaver fibroins. GA, GGX, and A_n form the consensus ensemble repeat units of silk fibroins from the pisaurid fishing spider, *Dolomedes*. The association of these three motifs in *Dolomedes* silk proteins mirrors the pattern seen in major and minor ampullate fibroins of orb-weavers (Fig. 3). GA, GGX, and A_n motifs are also distributed, sometimes sparsely, among ensemble repeat units from successively more basal lineages of spiders (Haplogynae and Mygalomorphae). A_n is represented in each of the fibroins from these taxa and from all lineages of Araneae studied thus far (Figs. 2 and 3). Mygalomorphae, tarantulas and their kin, diverged from Araneomorphae, "true" spiders, minimally

240 million years ago in the middle Triassic (*13*, Fig. 1), thus A_n motifs may have been maintained in different spider silks since that time.

Although the fibroins of *Plectreurys* (Haplogynae) and *Euagrus* (Mygalomorphae) are internally repetitive, the ensemble repeats from these basal taxa (Fig. 2) are unlike analogous units from previously described silks (Fig. 3). Each of the fibroins from these primitive groups contains stretches of serine. *Plectreurys* cDNA1 is highly internally repetitive with iterations of A_n, S_n, $(GX)_n$, and $(AQ)_n$. *Plectreurys* cDNA3 has a unique molecular architecture with the 5' end encoding a tandem array of long repeat units, and the 3' end encoding 15 repeats of a much shorter ensemble unit. The ~346 amino acid *Euagrus* repeat unit is a complex mixture of serine and alanine-rich sequences that includes a string of threonine, an amino acid that is rare in araneoid fibroins (Fig. 2).

Aside from an overall modular structure, scattered GA, GGX, and A_n motifs, and amino acid matches in the non-repetitive carboxy terminus, there is only limited sequence similarity between araneoid fibroins and those from *Plectreurys* and *Euagrus* (Figs. 2 and 3). Data presented herein clearly indicate that spiders utilize a broad diversity of fibroin sequences to spin silk threads, such diversity may be a reflection of the divergent ecosystems inhabited by these species (*1,14*). The novel fibroin repeats of basal Araneae suggest that spider silk design may not be especially dependent on specific sequences, but comparisons of fibroins among orb-weavers contradict this notion (Fig. 3).

In combination with published data (*4-5,8-9*), these new sequences allow comparisons between the two basal-most clades of ecribellate orb-weavers, Araneidae and "derived araneoids" (Fig. 1), for four groups of fibroins

(*15*): major ampullate spidroin 1-like (MaSp1), major ampullate spidroin 2-like (MaSp2), minor ampullate spidroins (MiSp), and flagelliform silk protein (Flag). Differences among fibroins within each of these four groups are primarily variations in the arrangement and frequency of A_n, GA, GGX, and $GPG(X)_n$ motifs (Fig. 3). A_n, GA, and GGX are present in consensus repeats for both araneid and derived araneoid MiSp orthologues. Major ampullate fibroins are similarly conserved among araneoids. Stable repeats for MaSp1 are A_n, GA, and GGX, and for MaSp2 are $GPG(X)_n$ and A_n. These motifs are retained even in major ampullate fibroins of the widow *Latrodectus*, a cob-web weaving araneoid that does not spin a conventional orb web. The long Flag repeats are divergent within Araneoidea, but both araneid and derived araneoid repeat units are comprised primarily of clustered $GPG(X)_n$ and GGX motifs (Fig. 3).

Fossil evidence suggests that the divergence of Araneidae from derived araneoids occurred no later than the early Cretaceous (Fig. 1). Therefore, the motifs conserved within MaSp1, MaSp2, MiSp, and Flag have been maintained, presumably by stabilizing selection, for over 125 million years (*16*). Motifs that have been retained over such long evolutionary periods are likely to be critical to the divergent mechanical properties of the specialized orb-weaver silks.

EXAMPLE II

Clones of MaSp1- and Masp2-like spider silk proteins

For the purposes of further classification and structure/function analyses of the novel spider silk proteins of the present invention, fibroin sequences were allocated to different ortholog groups of *Nephila*

clavipes. Silk fibroins are long proteins, comprised largely (>90%) of ensemble repeat units which are internally repetitive (4, 5, 8, 9). Ensemble repeat units from different fibroins vary in length, sometimes by an order of magnitude. It is difficult to make residue-to-residue homology statements between molecules because of this length variation and the overall modular structure of silk proteins. The gross similarities and differences between ensemble repeat units were, therefore, initially used to sort araneoid fibroins into four classes. The following proteins correspond to the MaSp1-like type of fibroin previously described in *N. clavipes* (5, 9).

MaSp1-like group proteins were characterized by short ensemble repeats with single polyalanine stretches. The remainder of a repeat was comprised of numerous GGX motifs and scattered GA motifs. The MaSp1-like group of spider silk proteins includes *N. clavipes* MaSp1, and proteins encoded by a genomic MaSp1 clone from *Argiope aurantia (A. aurantia)* (SEQ ID NO: 1), an *A. trifasciata* MaSp1 cDNA (SEQ ID NO: 2), a *Latrodectus geometricus (L. geometricus*) MaSp1 cDNA (SEQ ID NO: 3), a genomic MaSp1 clone from *N. madagascariensis* (SEQ ID NO: 4), a genomic MaSp1 clone from *N. senegalensis* (SEQ ID NO: 5), a genomic MaSp1 clone from *Tetragnatha kauaiensis (T. kauaiensis*) (SEQ ID NO: 6), and a genomic MaSp1 clone from *T. versicolor* (SEQ ID NO: 7). Amino acid sequences encoded by SEQ ID NOs: 1-7 are provided in SEQ ID NOs: 29-35, respectively.

MaSp2-like group proteins were characterized by short ensemble repeats comprised of one polyalanine stretch and various iterations of $GPG(X)_n$ and GP motifs. The MaSp2-like group of spider silk proteins includes *N. clavipes* MaSp2, and proteins encoded by a genomic MaSp2

clone from *A. aurantia* (SEQ ID NO: 8), an *A. trifasciata* MaSp2 cDNA (SEQ ID NO: 9), a genomic MaSp2 clone from *A. trifasciata* (SEQ ID NO: 10), a genomic MaSp2 clone from Gasteracantha mammosa (SEQ ID NO: 11), a *L. geometricus* MaSp2 cDNA (SEQ ID NO: 12), a genomic MaSp2 clone from *L. geometricus* (SEQ ID NO: 13), two genomic MaSp2 clones from *N. madagascariensis* (SEQ ID NOs: 14-15), and a MaSp2 genomic clone from *N. senegalensis* (SEQ ID NO: 16). Amino acid sequences encoded by SEQ ID NOs: 8-16 are provided in SEQ ID NOs: 36-44, respectively.

EXAMPLE III

Clones of flagelliform-like spider silk proteins

Based on the gross similarities and differences between ensemble repeat units, the following group of proteins was classified as flag-like type fibroins, similar to those previously described in *N. clavipes* (5, 9).

Flag-like group proteins were characterized by long ensemble repeats comprised mainly of clustered GGX and $GPG(X)_n$ motifs. Each ensemble repeat had a single "spacer" region that contained amino acids atypical of araneoid silks (5). The flag-like group of spider silk proteins includes Flag from *N. clavipes* and two proteins encoded by *A. trifasciata* Flag cDNA clones (SEQ ID Nos: 17-18). Amino acid sequences encoded by SEQ ID NOs: 17-18 are provided in SEQ ID NOs: 45-46, respectively.

EXAMPLE IV

Clones of spider silk proteins comprised of divergent motifs

Two of the novel spider silk proteins described herein could not be allocated readily into one of the

four classes of araneoid fibroins previously described in *N. clavipes*. This group of fibroins includes spider silk proteins comprised of divergent repetitive motifs, a feature which may reflect the diverse ecosystems of the species from which the nucleic acid sequences encoding these fibroins were derived. This category includes proteins encoded by a *Dolomedes tenebrosus* (*D. tenebrosus*) fibroin 1 cDNA (SEQ ID NO: 19) and a *D. tenebrosus* fibroin 2 cDNA (SEQ ID NO: 20). Amino acid sequences encoded by SEQ ID NOs: 19-20 are provided in SEQ ID NOs: 47-48, respectively.

EXAMPLE V

Clones of spider silk proteins comprised of atypical motifs

Seven novel spider silk proteins of the present invention comprise atypical spider silk motifs unlike those described for any previously characterized araneoid fibroin. This group of fibroins includes spider silk proteins comprised of divergent repetitive motifs, a feature which may reflect the diverse ecosystems of the species from which the nucleic acid sequences encoding these fibroins were derived. This category includes proteins encoded by an *Euagrus chisoseus* (*E. chisoseus*) fibroin 1 cDNA (SEQ ID NO: 21), a *Plectreurys tristis* (*P. tristis*) fibroin 1 cDNA (SEQ ID NO: 22), a *P. tristis* fibroin 2 cDNA (SEQ ID NO: 23), a *P. tristis* fibroin 3 cDNA (SEQ ID NO: 24), a *P. tristis* fibroin 4 cDNA (SEQ ID NO: 25), a *Phidippus audax* (*P. audax*) fibroin 1 cDNA (SEQ ID NO: 26), and a *Zorocrates* sp. fibroin 1 cDNA (SEQ ID NO: 27). Amino acid sequences encoded by SEQ ID NOs: 21-27 are provided in SEQ ID NOs: 49-55, respectively.

EXAMPLE VI

An exemplary clone of a spider silk protein comprised of divergent motifs

A novel spider silk protein of the present invention comprises atypical spider silk motifs unlike those described for any previously characterized araneoid fibroin. This fibroin is comprised of highly divergent repetitive motifs and is encoded by a *A. trifasciata* aciniform fibroin 1 cDNA clone (SEQ ID NO: 28). Amino acid sequences encoded by SEQ ID NO: 28 are provided in SEQ ID NO: 56.

A consensus sequence repeat of the *A. trifasciata* aciniform fibroin 1 protein (SEQ ID NO: 56) comprised of approximately 200 amino acids has been identified herein. Amino acid sequences comprising the consensus sequence repeat are provided in SEQ ID NO: 57. Such a consensus sequence is of use in a number of applications, including, but not limited to: 1) the generation of degenerative nucleic acid probes capable of encoding SEQ ID NO: 57 which can used to screen for and identify nucleic acid molecules encoding novel spider silk proteins, 2) the generation of antibodies specific for portions of or all of SEQ ID NO: 57 which can be used to screen for and identify novel spider silk proteins or derivatives thereof, and 3) utilization as a modular unit in the design and production of synthetic spider silk proteins.

EXAMPLE VII

Exemplary methods for designing synthetic spider silk proteins and uses thereof

The following methods for designing synthetic spider

silk proteins are based on the amino acid composition of spider silk proteins and how repetitive regions of amino acid sequences contribute to the structural/physical properties of spider silk proteins.

In general, synthetic spider silk proteins can be comprised of a series of tandem exact repeats of amino acid sequence regions identified as having a spectrum of physical properties. Exact repeats would comprise regions of amino acid sequences that are duplicated precisely. Alternatively, synthetic spider silk proteins can be comprised of a series of tandem inexact repeats identified as having a spectrum of physical properties. Inexact repeats would comprise regions of amino acid sequences in which at least one amino acid sequence can be altered in the basic inexact repeat unit as long as the alteration does not change the spectrum of physical properties characteristic of the basic inexact repeat unit.

In order to increase the tensile strength of minor ampullate silk for applications where strength and very little elasticity are needed, such as bulletproof vests, the (GA)n regions can be replaced by (A)n regions. This change would increase the tensile strength. The typical MiSp 1 protein has sixteen (GA) units. Replacing eight (GA) regions, for example, with (A) regions would increase the tensile strength from 100,000 psi to at least 400,000 psi. Moreover, if the (A)n regions were as long as the (GA)n regions the tensile strength would increase to greater than 600,000 psi.

To create a fiber with high tensile strength and greater elasticity than major ampullate silk, the number of (GPGXX) regions can be increased from 4-5 regions, which is the range of (GPGXX) regions typically found in naturally occurring major ampullate spider silk proteins, to a larger number of regions. For example, if the

number were increased to 10-12 (GPGXX) regions, the elasticity would increase to 50-60%. If the number were further increased to 25-30 regions, the elasticity would be near 100%. Such fibers can be used in coverings for wounds (for example, burn wounds) to facilitate easier placement and provide structural support. Such fibers can also be used for clothing and as fibers in composite materials.

The tensile strength of a very elastic flagelliform silk can be increased by replacing some of the (GPGXX) units with (A)n regions. A flagelliform silk protein contains an average of 50 (GPGXX) units per repeat. Replacing two units in each repeat with (A) regions can, therefore, increase the tensile strength of a flagelliform silk by a factor of four to achieve a tensile strength of about 400,000 psi. Uses for such flagelliform silk proteins are similar to those described for major ampullate proteins having augmented elasticity (as described hereinabove). The flagelliform proteins have additional utility in that the spacer regions therein confer the ability to attach functional molecules like antibiotics and/or growth factors (or combinations thereof) to composites comprising flagelliform proteins.

Fibers woven from combinations of the natural and/or synthetic spider silk proteins of the present invention are also encompassed herein. Such composite fibers have utility in a variety of applications, including, but not limited to, production of fabric, sutures, medical coverings, high-tech clothing, rope, and reinforced plastics.

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12. DNA sequences for the various fibroins were translated into amino acid sequences. For each fibroin, ensemble repeat units, higher-order aggregations of iterated sequence motifs, were aligned algorithmically then manually using MacVector (Oxford Molecular), and a consensus ensemble repeat unit was generated. The consensus ensemble repeat was defined as the most common amino acid (or gap) at each position in the alignment of repeats for each molecule. When there was a tie at an alignment position, X denoted this ambiguity in the consensus sequence.

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While certain of the preferred embodiments of the present invention have been described and specifically

exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A nucleic acid molecule encoding a protein of SEQ ID NO: 29 and naturally occurring allelic variants thereof.

2. A nucleic acid molecule encoding a protein of SEQ ID NO: 30 and naturally occurring allelic variants thereof.

3. A nucleic acid molecule encoding a protein of SEQ ID NO: 31 and naturally occurring allelic variants thereof.

4. A nucleic acid molecule encoding a protein of SEQ ID NO: 32 and naturally occurring allelic variants thereof.

5. A nucleic acid molecule encoding a protein of SEQ ID NO: 33 and naturally occurring allelic variants thereof.

6. A nucleic acid molecule encoding a protein of SEQ ID NO: 34 and naturally occurring allelic variants thereof.

7. A nucleic acid molecule encoding a protein of SEQ ID NO: 35 and naturally occurring allelic variants thereof.

8. A nucleic acid molecule encoding a protein of SEQ ID NO: 36 and naturally occurring allelic variants thereof.

9. A nucleic acid molecule encoding a protein of

SEQ ID NO: 37 and naturally occurring allelic variants thereof.

10. A nucleic acid molecule encoding a protein of SEQ ID NO: 38 and naturally occurring allelic variants thereof.

11. A nucleic acid molecule encoding a protein of SEQ ID NO: 39 and naturally occurring allelic variants thereof.

12. A nucleic acid molecule encoding a protein of SEQ ID NO: 40 and naturally occurring allelic variants thereof.

13. A nucleic acid molecule encoding a protein of SEQ ID NO: 41 and naturally occurring allelic variants thereof.

14. A nucleic acid molecule encoding a protein of SEQ ID NO: 42 and naturally occurring allelic variants thereof.

15. A nucleic acid molecule encoding a protein of SEQ ID NO: 43 and naturally occurring allelic variants thereof.

16. A nucleic acid molecule encoding a protein of SEQ ID NO: 44 and naturally occurring allelic variants thereof.

17. A nucleic acid molecule encoding a protein of SEQ ID NO: 45 and naturally occurring allelic variants thereof.

18. A nucleic acid molecule encoding a protein of SEQ ID NO: 46 and naturally occurring allelic variants thereof.

19. A nucleic acid molecule encoding a protein of SEQ ID NO: 47 and naturally occurring allelic variants thereof.

20. A nucleic acid molecule encoding a protein of SEQ ID NO: 48 and naturally occurring allelic variants thereof.

21. A nucleic acid molecule encoding a protein of SEQ ID NO: 50 and naturally occurring allelic variants thereof.

22. A nucleic acid molecule encoding a protein of SEQ ID NO: 51 and naturally occurring allelic variants thereof.

23. A nucleic acid molecule encoding a protein of SEQ ID NO: 52 and naturally occurring allelic variants thereof.

24. A nucleic acid molecule encoding a protein of SEQ ID NO: 53 and naturally occurring allelic variants thereof.

25. A nucleic acid molecule encoding a protein of SEQ ID NO: 54 and naturally occurring allelic variants thereof.

26. A nucleic acid molecule encoding a protein of SEQ ID NO: 55 and naturally occurring allelic variants thereof.

27. A nucleic acid molecule encoding a protein of SEQ ID NO: 56 and naturally occurring allelic variants thereof.

28. A nucleic acid molecule encoding a consensus silk protein sequence of SEQ ID NO: 56 and naturally occurring allelic variants thereof.

29. A nucleic acid selected from the group consisting of SEQ ID NOS: 1-28.

30. An expression vector comprising at least one of the nucleic acids of SEQ ID NOS: 1-28.

31. A host cell comprising the expression vector of claim 30.

32. A spider silk protein comprising SEQ ID NO: 29.

33. A spider silk protein comprising SEQ ID NO:30.

34. A spider silk protein comprising SEQ ID NO:31.

35. A spider silk protein comprising SEQ ID NO:32.

36. A spider silk protein comprising SEQ ID NO:33.

37. A spider silk protein comprising SEQ ID NO:34.

38. A spider silk protein comprising SEQ ID NO:35.

39. A spider silk protein comprising SEQ ID NO:36.

40. A spider silk protein comprising SEQ ID NO:37.

41. A spider silk protein comprising SEQ ID NO:38.

42. A spider silk protein comprising SEQ ID NO:39.

43. A spider silk protein comprising SEQ ID NO:40.

44. A spider silk protein comprising SEQ ID NO:41.

45. A spider silk protein comprising SEQ ID NO:42.

46. A spider silk protein comprising SEQ ID NO:43.

47. A spider silk protein comprising SEQ ID NO:44.

48. A spider silk protein comprising SEQ ID NO:45.

49. A spider silk protein comprising SEQ ID NO:46.

50. A spider silk protein comprising SEQ ID NO:47.

51. A spider silk protein comprising SEQ ID NO:48.

52. A spider silk protein comprising SEQ ID NO:49.

53. A spider silk protein comprising SEQ ID NO:50.

54. A spider silk protein comprising SEQ ID NO:51.

55. A spider silk protein comprising SEQ ID NO:52.

56. A spider silk protein comprising SEQ ID NO:53.

57. A spider silk protein comprising SEQ ID NO:54.

58. A spider silk protein comprising SEQ ID NO:55.

59. A spider silk protein comprising SEQ ID NO:56.

60. A spider silk amino acid consensus sequence comprising SEQ ID NO:57.

61. A spider silk protein selected from the group consisting of SEQ ID NOS: 29-56.

62. A silk fiber comprising at least one of the proteins of claim 61.

63. A copolymer fiber comprising at least two of the proteins of claim 61.

Figure 1

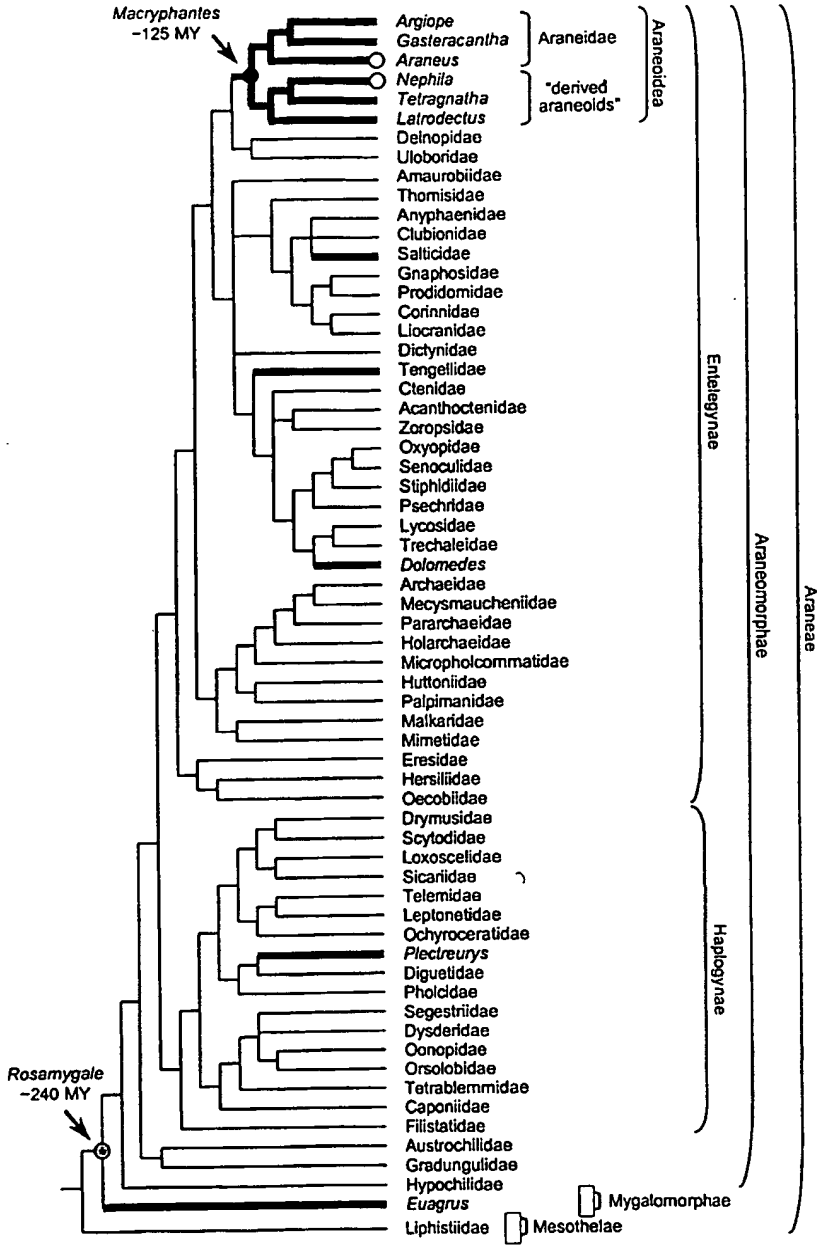
Macryphantes
~125 MY
Argiope
Gasteracantha
Araneus
Nephila
Tetragnatha
Latrodectus
Araneidae
"derived araneoids"
Araneoidea
Deinopidae
Uloboridae
Amaurobiidae
Thomisidae
Anyphaenidae
Clubionidae
Salticidae
Gnaphosidae
Prodidomidae
Corinnidae
Liocranidae
Dictynidae
Tengellidae
Ctenidae
Acanthoctenidae
Zoropsidae
Oxyopidae
Senoculidae
Stiphidiidae
Psechridae
Lycosidae
Trechaleidae
Dolomedes
Archaeidae
Mecysmaucheniidae
Pararchaeidae
Holarchaeidae
Micropholcommatidae
Huttoniidae
Palpimanidae
Malkaridae
Mimetidae
Eresidae
Hersiliidae
Oecobiidae
Drymusidae
Scytodidae
Loxoscelidae
Sicariidae
Telemidae
Leptonetidae
Ochyroceratidae
Plectreurys
Diguetidae
Pholcidae
Segestriidae
Dysderidae
Oonopidae
Orsolobidae
Tetrablemmidae
Caponiidae
Filistatidae
Austrochilidae
Gradungulidae
Hypochilidae
Euagrus
Liphistiidae
Rosamygale
~240 MY
Entelegynae
Haplogynae
Araneomorphae
Mygalomorphae
Mesothelae
Araneae

Figure 2

Dolomedes cDNA1
GGAGSGQGGYGNQGGLGGYGQGAGAGAAAAAAA

Dolomedes cDNA2
GGAGSGQGGYGGQGGLGGYGQGAGAGAAAAAAA

Plectreurys cDNA1
GAGAGAGAGAGAGAGSGASTSVSTSSSSGSGAGAGAGSGAGSGAGAGSGAGAGA
GAGGAGAGPGSGLGLGYGVGLSSAQAQAQAQAAAQAQAQAQAQAYAAAQAQAQAQAQ
AQAAAAAAAAAAA

Plectreurys cDNA2
TIAGLGYGRQGQGTDSSASSVSTSTSVSSSATGPDTGYPVGYYGAGQAEAAASAAAAAA
ASAAEAA

Plectreurys cDNA3
repeat type 1:
AISSSLYAFNYQASAASSAAAQSSAQTASTSAKQTAASTSASTAATSTTQTAATTSASTA
ASSQTVQKASTSSAASTAASKSQSSSVGSSTTSTAAASASSSYAFAQSLSQYLLSSQQFT
TAFASSTAVASSQQYAEAMAQSVATSLGLGYTYTSALSVAMAQAISGVGGGASAYSYAT
AISQAISRVLTSSGVSLSSSQATSVAS
repeat type 2:
SSQQSSYDTSSDLSSASSAAAAAASASSYESQFSDASSSSNAAAAA

Plectreurys cDNA4
SQQGPIGGVGGSNAFSSSFASALSLNRGFTEVISSASATAVASAFQKGLAPYGTAFALSA
ASAAADAYNSIGSGANAFAYAQAFARVLYPLVRQYGLSSSAKASAFASAIASSFSSGTSG
QGPSIGQQQPPVTISAASASAGASAAAVGGGQVGQGPYGGQQQSTAASASAAAATATS
GGAQKQPSGESSVATASAAATSVTSAGAPVGKPGVPAPIFYPQGPLQQGPAPGPSNVQ
PGT

Euagrus cDNA
ASQIAASVASAVASSASAAAAAASSSAAAAGASSAAGAASSSSTTTTTSTSSSAAAAAAA
AAAASASGASSASAAASASAAASAFSSALISDLLGIGVFGNTFGSIGSASAASSIASAAAQ
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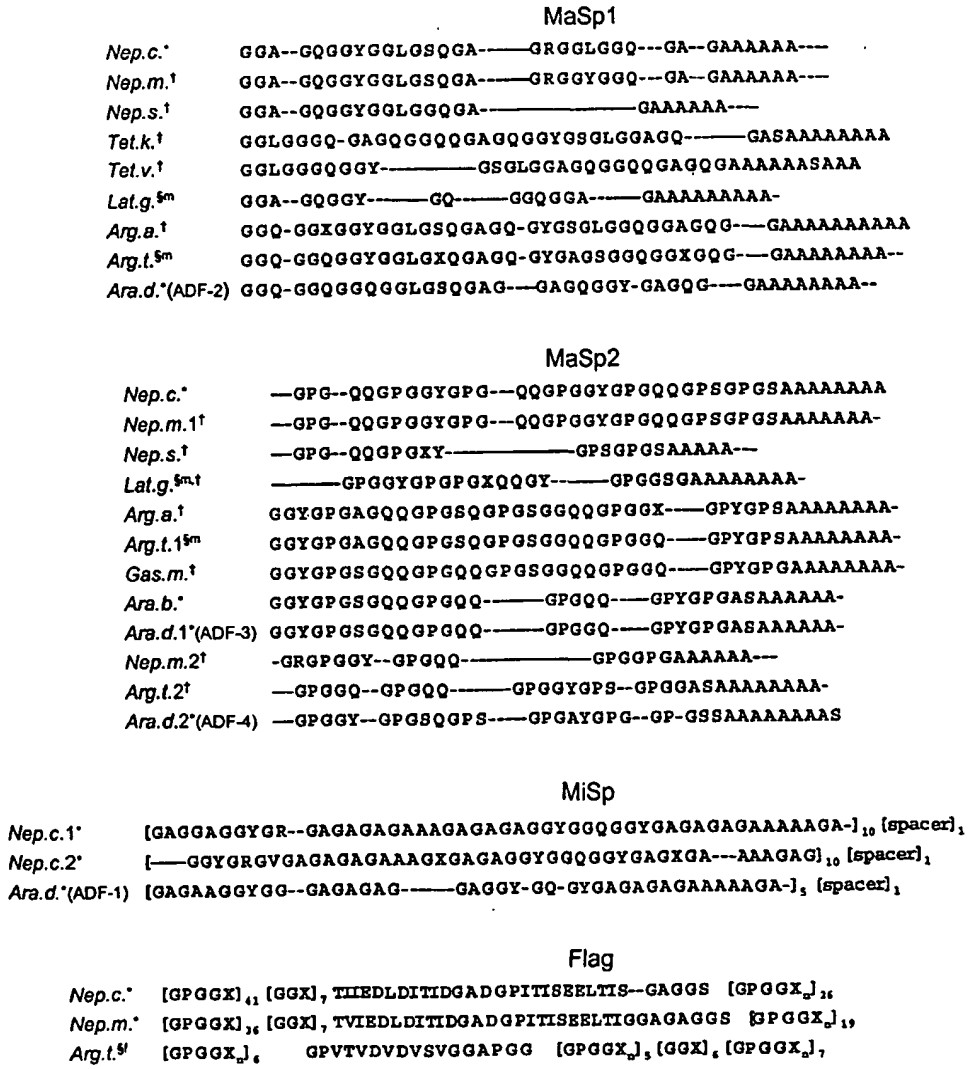
Figure 3


MaSp1
Nep.c.* GGA--GQGGYGGLGSQGA------GRGGLGGQ---GA--GAAAAAA----
Nep.m.† GGA--GQGGYGGLGSQGA------GRGGYGGQ---GA--GAAAAAA----
Nep.s.† GGA--GQGGYGGLGGQGA----------GAAAAAA----
Tet.k.† GGLGGGQ-GAGQGGQQGAGQGGYGSGLGGAGQ-------GASAAAAAAAAA
Tet.v.† GGLGGGQGGY----------GSGLGGAGQGGQQGAGQGAAAAAASAAA
Lat.g.§m GGA--GQGGY-------GQ-------GGQGGA-------GAAAAAAAAA-
Arg.a.† GGQ-GGXGGYGGLGSQGAGQ-GYGSGLGGQGGAGQG----GAAAAAAAAA
Arg.t.§m GGQ-GGQGGYGGLGXQGAGQ-GYGAGSGGQGGXGQG----GAAAAAAAA--
Ara.d.*(ADF-2) GGQ-GGQGGQGGLGSQGAG---GAGQGGY-GAGQG----GAAAAAAAA--
MaSp2
Nep.c.* —GPG--QQGPGGYGPG---QQGPGGYGPGQQGPSGPGSAAAAAAAA
Nep.m.1† —GPG--QQGPGGYGPG---QQGPGGYGPGQQGPSGPGSAAAAAAA-
Nep.s.† —GPG--QQGPGXY----------GPSGPGSAAAAA---
Lat.g.§m,† ———GPGGYGPGPGXQQGY-------GPGGSGAAAAAAAA-
Arg.a.† GGYGPGAGQQGPGSQGPGSGGQQGPGGX----GPYGPSAAAAAAAA-
Arg.t.1§m GGYGPGAGQQGPGSQGPGSGGQQGPGGQ----GPYGPSAAAAAAAA-
Gas.m.† GGYGPGSGQQGPGQQGPGSGGQQGPGGQ----GPYGPGAAAAAAAA-
Ara.b.* GGYGPGSGQQGPGQQ-------GPGQQ----GPYGPGASAAAAAA-
Ara.d.1*(ADF-3) GGYGPGSGQQGPGQQ-------GPGGQ----GPYGPGASAAAAAA-
Nep.m.2† -GRGPGGY--GPGQQ----------GPGGPGAAAAAA---
Arg.t.2† —GPGGQ--GPGQQ-------GPGGYGPS--GPGGASAAAAAAAA-
Ara.d.2*(ADF-4) —GPGGY--GPGSQGPS----GPGAYGPG--GP-GSSAAAAAAAAS
MiSp
Nep.c.1* [GAGGAGGYGR--GAGAGAGAAAGAGAGAGGYGGQGGYGAGAGAGAAAAAGA-]10 [spacer]1
Nep.c.2* [——GGYGRGVGAGAGAGAAAGXGAGAGGYGGQGGYGAGXGA---AAAGAG]10 [spacer]1
Ara.d.*(ADF-1) [GAGAAGGYGG--GAGAGAG------GAGGY-GQ-GYGAGAGAGAAAAAGA-]5 [spacer]1
Flag
Nep.c.* [GPGGX]41 [GGX]7 TIIEDLDITIDGADGPITISEELTIS--GAGGS [GPGGXn]16
Nep.m.* [GPGGX]36 [GGX]7 TVIEDLDITIDGADGPITISEELTIGGAGAGGS [GPGGXn]19
Arg.t.§† [GPGGXn]6 GPVTVDVDVSVGGAPGG [GPGGXn]5 [GGX]6 [GPGGXn]7

ADDENDUM I
Nucleic Acid Sequences

The following list of standard IUPAC nucleic acid one-letter abbreviations are provided for the purposes of clarity and define the nomenclature used to indicate ambiguities in nucleic acid sequences. The nucleic acid sequences of the present invention may include at least one ambiguous nucleic acid as indicated by the inclusion of one of the below listed one-letter abbreviations.

Y	pYrimidine	(C or T)
R	puRine	(A or G)
W	"Weak"	(A or T)
S	"Strong"	(C or G)
K	"Keto"	(T or G)
M	"aMino"	(C or A)
B	not A	(C or G or T)
D	not C	(A or G or T)
H	not G	(A or C or T)
V	not T	(A or C or G)
N	aNy base	(A or C or G or T)

SEQ ID NO: 1
Argiope aurantia major ampullate spidroin 1 (MaSp1) gene, partial cds.
Genbank Accession: AF350262
DNA sequence (1344 bp)

gagccggacaaggaggagctggagccgcagctgctgcagctgcagccggtggagctgga
ggtgctggaagaggaggattaggtgctggcggtgcaggacaaggatatggatccggatt
aggcggtcaaggaggagcaggtggtggcgctgccgcagctgcagcagcagcagcaggcg
gccaaggaggacaaggtggatatggcggattaggttctcaaggtgctggtcaaggagga
tatggagctggacaaggaggagctggagccgcagctgctgcagctgcagccggtggagc
tggaggtgctggaagaggaggattaggtgctggcggtgcaggacaaggatacggatccg
gattaggcggtcaaggaggagctggtcaaggtggtgctgccgcagcagcagcagcagct
ggcggccaaggaggacaaggtggatatggcggattaggttctcaaggtgctggtcaagg
aggagctggtcgtggcgctgccgcagccgcagcagcagctggcggccaaggaggacgag
gcggatatggcggattaggttctcaaggtgctggtcaaggaggatatggagctggacaa
ggaggagctggagccgcagctgctgcagctgcagccggtggagctggagaaggaggatt
aggtgctggcggtgcaggacaaggatatggatccggattaggcggtcaaggaggagctg
gtcaaggtggtgctgccgcagccgcagcagcagctggaggccaaggaggacatggtgga
tatggcggattaggttctcaaggtgctggtcaaggaggagctggtcgtggcgctgccgc
agccgcagcagcagctggcggtcaaggaggacagggtggatatggcggattaggttctc
aaggtgccggtcaaggaggatatggagctggacaaggaggagctgcagccgcagctgct
gcagctgcagccggtggagctggaggtgctggaagaggagaattaggtgctggcggtgc

aggacaaggatatggayccggattaggcggtcaaggaggagctggtcaacgtggtgccg
cttctgttgcagcattagctggagggcaaggaggacaaggtggttttggcggatttagt
tcacaaggagcaggtcaaggagcatatggtggtggtgcatacagtggacaaggagcagc
agcatctgtttccgctgcttccgctgcagcttcacgtctgtcatcacctggtgctgctt
cgagagtgtcttccgctgttacttctttggtatcaagtggcggcccaactaatcctgca
gctttatcgaatactatcagcartgttgtttctcaaattagtgaga

SEQ ID NO: 2
Argiope trifasciata major ampullate spidroin 1 (MaSp1)
mRNA, partial cds.
Genbank Accession: AF350266
DNA sequence (1947 bp)

gcagctgcagccgcagcagcagcagccggtggccaaggaggacaaggtggatatgacgg
attaggttctcaaggagccggtcaaggaggatacggacaaggaggagccgctgccgcag
cagccgcagccagtggagctggtagtgcccaacgaggaggcttaggtgctggaggtgca
ggacaaggatatggagccggatcaggcggtcaaggaggagctggacaaggtggcgcagc
tgcagccacagcagcagcagccggtggccaaggaggacaaggtggatatggcggattag
gttcccaaggatccggtcaaggaggatacggacaaggaggagccgctgccgcagcagcc
gcagccagtggagatggtggtgccggacaagaaggcttaggtgctggaggtgcaggaca
aggatatggtgctggattaggcggtcaaggaggagctggacaaggtggcgcagctgcag
ccgcagcagcagcagccggtggccaaggaggacaaggtggatatggcggattaggttct
caaggagccggtcaaggaggatacggacaaggaggagccgctgccgcagcagccgcagc
cagtggagctggtggcgccggacaaggaggcttaggtgctgcaggtgcaggacaaggat
atggtgccggatcaggcggtcaaggaggagctggacaaggtggcgcagctgcagctgca
gcagcagcagccggtggccaaggaggacaaggtggatatggcggattaggttctcaagg
agccggtcaaggaggatacggacaaggaggagtcgctgctgcagcagccgcagccagtg
gagctggtggtgccggacgaggaggcttaggtgctggaggtgcaggacaagaatatggt
gccgtatcaggcggtcaaggaggagctggacaaggtggcgaagctgcagccgcagcagc
agcagccggtggccaaggaggacaaggtggatatggcggattaggttctcaaggagccg
gtcaaggaggatacggacaaggaggagccgctgccgcagcagcagcagccagtggagct
ggtggtgccagacgaggaggcttaggtgctggaggtgcaggacaaggatatggtgccgg
attaggtggtcaaggaggagcaggacaaggtagcgcatctgcagccgcagcagcagcag
ccggtggccaaggaggacaaggtggatatggcggattaggttctcaaggatccggtcaa
ggaggatacggacaaggaggagccgctgccgcagcagccgcagccagtggagctggtgg
tgccggacgaggaagcttaggtgctggaggtgcaggacaaggttatggtgctggattag
gcggtcaaggaggagctggacaaggtggcgcagctgcagccgcatcagcagcagccggt
ggccaaggaggacaaggtggatatggcggattaggttctcaaggagctggtcaaggagg
atacggacaaggaggagccgctgccgcagcagcatcagccggtggccaaggagggcaag
gtggatatggtggattaggttctcaaggagccggtcagggaggatatggtggtggggca
ttcagtggccaacaaggcggagcagcatctgttgccactgcttccgctgctgcttcacg
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attagttcaagcaatcctggtctctctggctgtgatgttcttgttcaagcattacttga
aattgtttcagctttggtacatattcttggttcagctaacattggacaagttaactcca
gcggtgttgggcgatcagcttctattgtgggacaatctataaaccaagctttctcataa

SEQ ID NO: 3
Latrodectus geometricus major ampullate spidroin 1 (MaSp1) mRNA,partial cds.
Genbank Accession: AF350273
DNA sequence (1083bp)

gctggctcaggacaaggtggttatggacaaggatatggtgaaggtggtgctggacaaggg
ggagcaggagccgcagcagcagccgctgcagcagctggtggagctggacaaggtggacaa
ggcggttatggacaaggatatggtcaaggtggtgccggacaaggtggagcaggagccgca
gcagcagctgcagctggtggagctggacaaggaggctacggccgaggtggagcaggacaa
ggtgcagcagcggcagcagcagctgcaggttcaggacaaggaggacaaggtggttatgga
caaggttatggtcaaggtggtgctggacaaggggggagcaggagccgcagcagcagcagct
gcagctggtggagctggacaaggaggatacggacgaggaggagcaggacagggaggagca
gctgcagccgctgcagcagctggaggagccggtcaaggtggacaaggcggttatggacaa
ggatatggtcaaggtggtgccggacagggtggagcaggagccgcagcagcagcagctgca
gctggtggagctggacaaggaggatatggccgaggtggagcaggacaaggtggatcagca
gcagccgcagcagcagctggtggagcaggacaaggaggatatggccgaggtggtgccgga
caaggtggagcaggttcagcagcggcagcagctgcagctggcggttctggacaaggagga
caaggtggttatggacaaggatatggtcaaggtggtgctggacaaggtggagcagctgct
gcagcttctgctttggcagctccagctacaagtgcgagaatttcttctcatgcctcgact
cttctttcaaatggtcccaccaatccagcttcaatttcaaatgttattagtaatgctgta
tcccaaattagttcgagcaatccaggagcttcttcgtgtgacgttcttgttcaagctctt
cttgaacttgtcacagcgttactcaccattattgggtcctctaatgttggcaatgttaat
tatgattcttcaggccaatatgcacaagtggtttcacagtccgtgcaaaacgcatttgtt
taa

SEQ ID NO: 4
Nephila madagascariensis major ampullate spidroin 1 (MaSp1) gene,partial cds.
Genbank Accession: AF350277
DNA sequence (703 bp)

gaggtcttggtggacaaggtgcaggacaaggagctggagcagcagcagcagctggtg
gtgccggacaaggaggatatggaggtcttggaagccaaggtgctggccgaggcggatatg
gtggacaaggagctggagcagcagcagccgctgccgcaggaggtgccggacaaggaggat
atggaggtcttggaagccaaggtgctggacaaggaggatacggaggtcttggtggacaag
gtgcaggacaaggagcagcagcagcagcagctggtggtgccggacaaggaggatatg
gaggtcttggaagccaaggtgctggccgaggcggatatggtggacaaggtgcaggagcag
cagcagctgcaactggtggtgctggacaaggaggatatggtggtgtcggttctggggcgt
ctgctgcctctgcagctgcatcacgtttgtcttctcctcaagctagttcaagagtttcat
cagctgtttccaacttggttgcaagtggtcctacgaattctgcggcattgtcaagtacaa
tcagtaacgcggtttcacaaattggcgccagcaatcctggtctttctggatgtgatgtcc
tcattcaagctcttctcgaggttgtttctgctcttatccatatcttaggttcttccagca
tcggccaagttaattatggttccgctggtcaagccactcagat

SEQ ID NO: 5
Nephila senegalensis major ampullate spidroin 1 (MaSp1) gene, partial cds.
Genbank Accession: AF350279
DNA sequence (763 bp)

gaggtcttggtggacaaggtgctggacgaggagctggagcagccgctgcagcagctggag
gtgctggacaaggaggatacggaggtcttggtggacaaggagctggagccgctgccgcag
cagcgggtggtgccggacaaggaggacaaggattaggtggaagaggtgcagcagcagctg
gaggtgctggacaaggaggatacggaggtcttggtggacaaggtgctggacgaggagctg
gagcagccgctgcagcagctggaggtgctggtcaaggaggatacggaggtcttggtggac
aaggagctggagcagcagcagcagccgctgcagcaggaggtgctggacaaggagggtatg
gaggtcttggaagccaaggtgctggacgaggaggatatggaggacaaggtgcaggagcgg
cagtagcagcgattggtggcgttggacaaggaggctatggtggtgtcggttctggggcgt
ctgctgcctctgcagctgcttctcgcttgtcttctcccgaagctagttcaagagtttcat
ctgctgtttccaacttggtttcaagtggtcctactaattctgcggcattgtcaagtacta
tcagtaatgtggtctcacaaataggcgccagcaatcctggtctttctggatgtgatgtcc
tcattcaagctcttctcgaagttgtttctgctcttgtccatatcttaggctcttccagca
tcggccaggttaattatggttccgctggtcaagccactcagat

SEQ ID NO: 6
Tetragnatha kauaiensis major ampullate spidroin 1 (MaSp1) gene,partial cds.
Genbank Accession: AF350285
DNA sequence (853 bp)

gatccggactcggaggagcaggacaaggagccggccaaggagcatcagctgccgccgcmg
cagcagcagsaggaggccttggaggtggccaaggagcaggtcaaggaggacaacaaggtg
cyggacaaggaggctacggatccggactcggaggagcaggacaaggagcatcagctgccg
ccgcagcagcagcaggaggccttggaggtggccaaggagcaggtcaaggaggacaac
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caagaaactccgcaactaatgcaatttctaattctgcctctaacgctgtctcacttctct
catcacctgcttcaaatgcaagaatttcttctgctgtgtctgccttggcatccggtgcag
catctggtcctggatatttatctagcgttatcagtaatgttgtttctcaagtcagctcaa
acagtggtggacttgttggttgcgatactcttgttcaagctcttcttgaagctgctgctg
ctcttgtgcatgtattggcttcttctagtggtggacaagtcaaccttaacacagcgggat
acacttctcaact

SEQ ID NO: 7
Tetragnatha versicolor major ampullate spidroin 1 (MaSp1) gene,partial cds.
Genbank Accession: AF350286
DNA sequence (535 bp)

gatctggacaaggagcatccgccgctgcggcagcagcaggaggccttggaggtggacaag
gaggttacggttctggtctaggaggtgcaggacaaggaggacaacaaggagctggacaag
gagcagcagctgccgcagcatcagcagcagcaggaggccttggaggtggacaaggaggtc
aacaaggagcaggccgaggtggactacaaggagctggacaaggaggacaaggtgctctag
gtggatcaagaaactccgcagctaatgcagtttcacgtctctcttcacctgcttcaaatg
caagaatttcttctgctgtgtctgccttggcatccggtggagcatctagtcccggatatt
tatctagcattattagcaatgtggtttctcaggttagctcaaacaatgatggactttctg
ggtgcgacactgttgttcaagctcttcttgaagttgctgctgctcttgtgcatgtattgg
cttcttctaatattgggcaagtcaaccttaatactgccggatacacttcccaact

SEQ ID NO: 8
Argiope aurantia major ampullate spidroin 2 (MaSp2) gene, partial cds.
Genbank Accession: AF350263
DNA sequence (1049 bp)

accaggmggtgccggccaacaaggtcctggcggtcaaggaccatacggaccaggtgcag
ccgccgcagcagcagccgctggaggatatggaccaggagctggacaacaaggcccagrt
ggagccggacaacaaggacccggwtcccaaggaccaggaggtgccggtcaacaaggacc
tggtggacaaggaccatacggaccaggagcagccgccgcagcagcagcagtaggaggat
ayggaccaggagctggacaacaaggacctggaagtcaaggaccaggaagtggtggacaa
caaggacctggtggtcaaggaccttatggaccaagtgcagccgccgcagcagcagccgc
tggaggctatggaccaggagctggacaacaaggacctggaagtcaaggaccaggaagtg
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tgcagccgcagcagcagcagccgctggaggctatggaccaggagctggacaacaaggac
ctggaagtcaggcaccagttgcatccgcagcagcctctcgtctttcttctcctcaagcc
agttctagagtttcatctgctgtgtcaactttggtgtcgagtggtcctacgaatcctgc
cgcactttctaatgctatcagtagcgttgtatcacaagttagtgcaagtaatcctggtc
tttctggttgtgacgttctcgttcaagcattgctggaacttgtatccgcccttgtacac
atccttgggtcttccagcattgggcaaattaattacgccgcgtctt

SEQ ID NO: 9
Argiope trifasciata major ampullate spidroin 2 (MaSp2) mRNA, partial cds.
Genbank Accession: AF350267
DNA sequence (1336 bp)

cgctggaccaggatacggaccaggagccggacaacaaggacctggaagtcaaggaccag
gaagtggtggacaacaaggacctggtggacaaggaccatatggaccaagcgctgccgcc
gcagcagctgccgctggaccaggatatggaccaggagctggacaacaaggaccaggaag
tggcggacaacaaggaggccaaggatctggacagcaaggaccaggaggtgccggtcaag
gaggtcctcgtggtcaaggaccatacggaccaggtgcagccgccgccgccgcagctgct
ggaggatacggaccaggagctggacaacaaggacctggaagtcaaggacccggaagtgg
tggacaacaaggtcctggtagtcaaggaccatatggaccaagtgcagccgcagcagcag
cagccgctggaccaggatacggaccaggagccggacaacaaggacctggaagtcaagga
ccaggaagtggtggacaacaaggacctggtggacaaggaccatatggaccaagcgatgc
cgccgcagcagctgccgctggaccaggatatggaccaggagctggacaacaaggaccag
gaagtggcggacaacaaggaggccaaggatctggacagcaaggaccaggaggtgccggt
caaggaggtcctcgtggtcaaggaccatacggaccaggtgcagccgccgccgccgcagc
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tatccttggatcttctagcattgggcaaattaattacgccgcttcttctcagtatgcgc
aattggttggtcaatctttaactcaagcccttggttga

SEQ ID NO: 10
Argiope trifasciata major ampullate spidroin 2-like protein gene,partial cds.
Genbank Accession: AF350268
DNA sequence (360 bp)

ggacctggacaacaaggacctggagggtatggaacatccggacctggaggtgcttctgc
cgccgccgctgctgcagctgcaggtggacctggaggacaaggaccatctggaccaggac
caccaggaccaggaggatatggaccatccggaccaggagcagccgcagccgccgctgca
gcagcaggtggacccggaagtcaaggacctggacaacaaggacccggaggctacggacc
atctggacctggaggagcttctgccgccgccgctgctgcagctgcaggtggacccggag
gtcaaggatcatacggaccaggacaacaaggaccaggtgcaggacaatacggacccgga
caacag

SEQ ID NO: 11
Gasteracantha mammosa major ampullate spidroin 2 (MaSp2) gene, partial cds. (note: some consider G. mammosa a subspecies of G. cancriformis)
Genbank Accession: AF350272
DNA sequence (1026 bp)

ggccaacaaggacctggaagtcaaggaccatacggacctggtgcagcagctgccgcagc
agcagcagctggaggataccgacctgtatctggtcaacaaggacctggacaacaaggac
caggaagcggtggccaacaaggacctggaggccaacgaccttacggaccaggtgcagcc
gcagcagcagcagccgcaggaggatacggacctggatctggacaaggaggacctggaca
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acaaggtccatacggacctagtgccgctgcagcagcagcagccgttggaggatacggac
caggagctggacagcaaggacctggacaacaaggaccaggaagtggtggccaacgagga
cctggaggtcaaggaccatatggaccaggagcagcagctgccgcagcagcagcagctgg
tggatatggacctgcatctggtcaacaaggacctggacaacaaggaccaggaagtggtg
gccaacgaggacctggaggtcaaggaccatatggaccaggtgcagcagcagcagcatct
gcaggaggatatggaccaggaagtggtggaagccctgcatcaggagcagcttctcgact
ttcttctcctcaagccggtgccagagtttcttcagctgtatcagcccttgtcgcaagtg
gcccaactagtccagctgctgtttccagcgccatcagtaatgttgcatcacaaattagt
gcaagcaatcctggtctttccggctgcgatgttcttgtacaagcattacttgagattgt
atcagctcttgtatctattctctcatccgctagtatcggacaaatcaattatggcgcat
ctggtcaatatgccgccatgatt

SEQ ID NO: 12
Latrodectus geometricus major ampullate spidroin 2 (MaSp2) mRNA, partial cds.
Genbank Accession: AF350274
DNA sequence (1118bp)

gcatctgcgtctggtggagcaggacctggaagacaacaaggatatggaccaggaggatca
ggagcctcggcagcagcagccgccgccgctggaggagctggcccaggaggatacggacaa
ggaccatctggttacggcccatctggacctggtgcacaacaaggttacggaccaggaggc
caaggaggatctggagcagcagctgcagcagccgcagcagcaggctctggacctggagga
tatggaccaggagcagcaggaccaggaagttatggtccaagtggacctggaggatctggt
gcagctgccgcagccgctgctgctagtggaccaggaggacaacaaggatatggaccagga
ggaccaggagcctcagcagcagcagccgccgccgctggaggatctggacctggaggatac
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ggaggccaaggaggatctggagcagcagctgcagcagccgcagcagcaggctctggacgt
ggaggatatggaccaggagcagcaggaccaggaaattatggtccaagtggacctggagga
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ccaggtggatctggagcagctgctgcagccgcgtctggtggagcaggacctggaagacaa
caaggatatggaccaggaggatcaggagccgcagcagcagcagccgccgccgctggagga
tctggtccaggaggatacggacaaggaccagccggttacggaccaggaggccaaggagga
tccggaggagcagctgcagcagccgcagcagcaagctctggacccggaggatatggacca

ggagcagcaggaccaggcaattatggtccaagtggacctgggggatctggtgcagctgcg
gcagctgctgctgctagtggaccaggaggacaacaaggatacggaccaggtggatctggt
gcatctgcagcagcagcggctggtggtgcaggacctggaagacaacaagcatatggacct
ggaggatcaggagctgcagcagcagcagcgagtggatc

SEQ ID NO: 13
Latrodectus geometricus major ampullate spidroin 2 (MaSp2) gene, partial cds.
Genbank Accession: AF350275
DNA sequence (1196 bp)

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SEQ ID NO: 14
Nephila madagascariensis major ampullate spidroin 2-like protein gene, partial cds.
Genbank Accession: AF350276
DNA sequence (5858 bp)

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SEQ ID NO: 15
Nephila madagascariensis major ampullate spidroin 2 (MaSp2) gene, partial cds.
Genbank Accession: AF350278
DNA sequence (1692 bp)

aacagggaccatctggacctggaagtgcagcggcagcggcagcagcaggacctggacaac
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SEQ ID NO: 16
Nephila senegalensis major ampullate spidroin 2 (MaSp2) gene, partial cds.
Genbank Accession: AF350280
DNA sequence (693 bp)

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SEQ ID NO: 17
Argiope trifasciata flagelliform silk protein (Flag) mRNA, partial cds.(with C-term.)
Genbank Accession: AF350264
DNA sequence (1958 bp)

gtgcaggtggaccaggagcaggtggagcaggagctggtggtgtcggacctggaggattt
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SEQ ID NO: 18
Argiope trifasciata flagelliform silk protein (Flag) mRNA,
partial cds. (without C-term.)
Genbank Accession: AF350265
DNA sequence (3008 bp)

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SEQ ID NO: 19
Dolomedes tenebrosus fibroin 1 mRNA, partial cds.
Genbank Accession: AF350269
DNA sequence (2565 bp)

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Genbank Accession: AF350270
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DNA sequence (2740 bp)

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Genbank Accession: AF350282
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SEQ ID NO: 25
Plectreurys tristis fibroin 4 mRNA, partial cds.
Genbank Accession: AF350284
DNA sequence (5446bp)

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SEQ ID NO: 26
Phidippus audax fibroin 1 mRNA, partial cds.
DNA sequence (1711 bp)

ggagctggagctggcgctggctatggtgcaggtgctggttcaggagctggtgcaggctc
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SEQ ID NO: 27
Zorocrates sp. fibroin 1 mRNA, partial cds.
DNA sequence (845 bp)

ggtgcagcagccgcagcctcagcagcagcagcaggcggacgaggaagccaaggaggtta
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SEQ ID NO: 28
Argiope trifasciata aciniform fibroin 1 mRNA, partial cds.
DNA sequence of 3' region (709 bp)

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ADDENDUM II
Amino Acid Sequences

SEQ ID NO: 29
Amino acid sequences encoded by SEQ ID NO: 1

AGQGGAGAAAAAAAAGGAGGAGRGGLGAGGAGQGYGSGLGGQGGAGGGAAAAAAAAAGG
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SVAALAGGQGGQGGFGGFSSQGAGQGAYGGGAYSGQGAAASVSAASAAASRLSSPGAAS
RVSSAVTSLVSSGGPTNPAALSNTISXVVSQISE

SEQ ID NO: 30
Amino acid Sequences encoded by SEQ ID NO: 2

AAAAAAAAAGGQGGQGGYDGLGSQGAGQGGYGQGGAAAAAAAASGAGSAQRGGLGAGGA
GQGYGAGSGGQGGAGQGGAAAATAAAAGGQGGQGGYGGLGSQGSGQGGYGQGGAAAAAA
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SEQ ID NO: 31
Amino acid sequences encoded by SEQ ID NO: 3

AGSGQGGYGQGYGEGGAGQGGAGAAAAAAAAAGGAGQGGQGGYGQGYGQGGAGQGGAGAA
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SEQ ID NO: 32
Amino acid sequences encoded by SEQ ID NO: 4

GLGGQGAGQGAGAAAAAAGGAGQGGYGGLGSQGAGRGGYGGQGAGAAAAAAAGGAGQGGY
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SNAVSQIGASNPGLSGCDVLIQALLEVVSALIHILGSSSIGQVNYGSAGQATQ

SEQ ID NO: 33
Amino acid sequences encoded by SEQ ID NO: 5

GLGGQGAGRGAGAAAAAAGGAGQGGYGGLGGQGAGAAAAAAGGAGQGGQGLGGRGAAAAG
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AVSNLVSSGPTNSAALSSTISNVVSQIGASNPGLSGCDVLIQALLEVVSALVHILGSSSI
GQVNYGSAGQATQ

SEQ ID NO: 34
Amino acid sequences encoded by SEQ ID NO: 6

SGLGGAGQGAGQGASAAAAAAAXGGLGGGQGAGQGGQQGAGQGGYGSGLGGAGQGASAAA
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SGGLVGCDTLVQALLEAAAALVHVLASSSGGQVNLNTAGYTSQL

SEQ ID NO: 35
Amino acid sequences encoded by SEQ ID NO: 7

SGQGASAAAAAAGGLGGGQGGYGSGLGGAGQGGQQGAGQGAAAAAASAAAGGLGGGQGGQ
QGAGRGGLQGAGQGGQGALGGSRNSAANAVSRLSSPASNARISSAVSALASGGASSPGYL
SSIISNVVSQVSSNNDGLSGCDTVVQALLEVAAALVHVLASSNIGQVNLNTAGYTSQL

SEQ ID NO: 36
Amino acid sequences encoded by SEQ ID NO: 8

PGGAGQQGPGGQGPYGPGAAAAAAAAGGYGPGAGQQGPXGAGQQGPGSQGPGGAGQQGPG
GQGPYGPGAAAAAAAVGGYGPGAGQQGPGSQGPGSGGQQGPGGQGPYGPSAAAAAAAAGG
YGPGAGQQGPGSQGPGSGGQQGPGGLGPYGPSAAAAAAAAGGYGPGAGQQGPGSQGPGSG
GQQRPGGLGPYGPSAAAAAAAAGGYGPGAGQQGPGSQGPGSGGQQRPGGLGPYGPSAAAA
AAAAGGYGPGAGQQGPGSQAPVASAAASRLSSPQASSRVSSAVSTLVSSGPTNPAALSNA
ISSVVSQVSASNPGLSGCDVLVQALLELVSALVHILGSSSIGQINYAAS

SEQ ID NO: 37
Amino acid sequences encoded by SEQ ID NO: 9

AGPGYGPGAGQQGPGSQGPGSGGQQGPGGQGPYGPSAAAAAAAAGPGYGPGAGQQGPGS
GGQQGGQGSGQQGPGGAGQGGPRGQGPYGPGAAAAAAAAGGYGPGAGQQGPGSQGPGSG
GQQGPGSQGPYGPSAAAAAAAAGPGYGPGAGQQGPGSQGPGSGGQQGPGGQGPYGPSDA
AAAAAAGPGYGPGAGQQGPGSGGQQGGQGSGQQGPGGAGQGGPRGQGPYGPGAAAAAAA
AGGYGPGAGQQGPGSQGPGSGGQQGPGSQGPYGPSAAAAAAAAGPGYGPGAGQQGPGSQ
GPGSGGQQGPGSQGPYGPSAAAAAAAAGPGYGPGAGQQGPGSQAPVASAAASRLSSPQA

SSRVSSAVSTLVSSGPTNPASLSNAISSVVSQVSSSNPGLSGCDVLVQALLEIVSALVH
ILGSSSIGQINYAASSQYAQLVGQSLTQALG

SEQ ID NO: 38
Amino acid sequences encoded by SEQ ID NO: 10

GPGQQGPGGYGTSGPGGASAAAAAAAAGGPGGQGPSGPGPPGPGGYGPSGPGAAAAAAA
AAGGPGSQGPGQQGPGGYGPSGPGGASAAAAAAAAGGPGGQGSYGPGQQGPGAGQYGPG
QQ

SEQ ID NO: 39
Amino acid sequences encoded by SEQ ID NO: 11

GQQGPGSQGPYGPGAAAAAAAAAGGYRPVSGQQGPGQQGPGSGGQQGPGGQRPYGPGAAA
AAAAAGGYGPGSGQGGPGQQGPGSGGQQGPGGQGPYGPGAAAAAAAAAGGYGPGSGQGGQ
QGPGSQGPGSGGQQGPGGQGPYGPSAAAAAAAVGGYGPGAGQQGPGQQGPGSGGQRGPGG
QGPYGPGAAAAAAAAAGGYGPASGQQGPGQQGPGSGGQRGPGGQGPYGPGAAAAASAGGY
GPGSGGSPASGAASRLSSPQAGARVSSAVSALVASGPTSPAAVSSAISNVASQISASNPG
LSGCDVLVQALLEIVSALVSILSSASIGQINYGASGQYAAMI

SEQ ID NO: 40
Amino acid sequences encoded by SEQ ID NO: 12

ASASGGAGPGRQQGYGPGGSGASAAAAAAAGGAGPGGYGQGPSGYGPSGPGAQQGYGPGG
QGGSGAAAAAAAAAGSGPGGYGPGAAGPGSYGPSGPGGSGAAAAAAAAASGPGGQQGYGPG
GPGASAAAAAAAGGSGPGGYGQGPSGYGPSGPGAQQGYGPGGQGGSGAAAAAAAAAGSGR
GGYGPGAAGPGNYGPSGPGGSGAAASAAAASGPGGQQGYGPGGSGAAAAAASGGAGPGRQ
QGYGPGGSGAAAAAAAAAGGSGPGGYGQGPAGYGPGGQGGSGGAAAAAAAASSGPGGYGP
GAAGPGNYGPSGPGGSGAAAAAAAASGPGGQQGYGPGGSGASAAAAAGGAGPGRQQAYGP
GGSGAAAAAASGS

SEQ ID NO: 41
Amino acid sequences encoded by SEQ ID NO: 13

AGPGSYGPSGPGGSGAAAAAAAASGPGGQQGYGPGGPGASAAAAAAAGGSGPGGYGQGPS
GYGPSGPGAQQGYGPGGQGGSGAAAAAAAAAGSGPGGYGPGAAGPGNYGPSGPGGSGAAA
SAAAASGPGGQQGYGPGGSGAAAAAASGGAGPGRQQGYGPGGSGAAAAAAAAXGGSGPGG
YGQGPXGYGPGGQGGSGGAAAAAAAAASSGPXGYGPGAAGPGNYGPSGPGGSGAAAAAAAA
SGPGGQQGYGPGGSGASAAAAAGGAGXGRQQAYGPGGSGAAAAAASGSGGYGPAQYGXSS
VASSAASAASALSSPTTHARISSHASTLLSSGPTNSAAISNVISNAVSQVSASNPGSSSC
DVLVQALLELITALISIVDSSNIGQVNYGSSGQYAQMVG

SEQ ID NO: 42
Amino acid sequences encoded by SEQ ID NO: 14

GSYGQGPSGYAQGSSAASAAAPSGYVPSQTGQSGLGAAAAAAAVAPSGYGPSQQGPSGPG
AATAAAAGRGPEGYGPRQQGPGATAAAAGPGGYGPRQQGPGGYGPGQQGPGAAAAAAAGR

GPGGYGPGQQGPGGPGAAAAAAGSEGYGPGQQGPRGPGAAAAGPGGYGPGQQGASAAASA
AAGRGPGGYGPGQQGPGGPSAAAAGPGGYGPGQQGPSAAAAAAAGSGPGGYGPGQQGPGG
PGAAAAAAGPGGYGPGQQGPGAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAAGRGPGGYG
PGQQGPGGPGAAAAAAGPGGYGPGGYGPGQQGPGGPGAAAAAAAGRGPGGYGPGQQGPGQ
QGPGGSGAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAGPGGYGPGQQGPGAAAAAAAAGR
GPGGYGPGQQGPGGPGAAAAAAAGRGPGGYGPGQQGPGQQGPGGSGAAAAAAGRGPGGYG
PGQQGPGGPGAAAAAAGPGGYGPGQQGPGAAAAAAAAGRGPGGYGPGQQGPGGSGAAAAA
AGRGPGGYGPGQQGPGGPGAAAAAAGPGGYGPGQQGTGAAAAAAAGSGAGGYGPGQQGPG
GPGAAAAAAGPGGYGPGQQGPGAAAAAAAGSGPGGYGPGQQGPGGSSAAAAAAGPGRYGP
GQQGPGAAAAASAGRGPGGYGPGQQGPGGPGAAAAAAGPGGYGPGQQGPGAAAAAAAGSG
PGGYGPGQQGPGGPGAAAAAAAGRGPGGYGQGQQGPGGPGAAAAAAGPGGYGPGQQGPGA
AAAAAAGSGPGGYGPGQQGPGRSGAAAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAGPGG
YGPGQQGPGAAAAASAGRGPGGYGPGQQGPGGSGAAAAAAGRGPGGYGPGQQGPGGPGAA
AAAAAGRGPGGYGPGQQGPGQQGPGGSGAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAGP
GGYGPGQQGPGAAAAAAAGSGPGGYGPGQQGPGGPGAAAAAAGRGPGGYGPGQQGPGGPG
AAAAAAAGRGPGGYGPGQQGPGQQGPGGSGAAAAAAGRGPGGYGPGQQGPGGPGAAAAAA
GPGGYGPGQQGPGAAAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAAGRGPGGYGPGQQGP
GQQGPGGPGAAAAAAGPGGYGPGQQGTGAAAAAAAGSGAGGYGPGQQGPGGPGAAAAAAG
PGGYGPGQQGPGAAAAAAAGSGPGGYGPGQQGPGGSSAAAAAAGPGRYGPGQQGPGAAAA
AAAGSGPGGYGPGQQGPGGPGAAAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAGPGGYGP
GQQGPGAAAAAAAGSGPGGYGPGQQGPGGPGAAAAAAAGRGPGGYGQGQQGPGGPGAAAA
AAGPGGYGPGQQGPGAAAAAAAGSGPGGYGPGQQGPGRSGAAAAAAAAGRGPGGYGPGQQ
GPGGPGAAAAAAGPGGYGPGQQGPGAAAAASAGRGPGGYGPGQQGPGGSGAAAAAAGRGP
GGYGPGQQGPGGPGAAAAAAAGTGPGGYGPGQQGPGGSGAAAAAAGRGPGGYGPGQQGPG
GPGAAAAAAGPGGYGPGQQGPGAAAAAAAGSGPGGYGPGQQGPGGPGAAAAAAGRGPGGY
GPGQQGPGGPGAAAAAAAGRGPGGYGPGQQGPGGSGAAAAASGRGPGGYGPGQQGPGGPG
AAAAAAGPGGYGPGQQGPGAAAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAAGRGPGGYG
PGQQGPGGSGAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAGPGGYGPGQQGPGAAAAAAG
RGPGGYGPGQQGPGGSGAAAAAAGRGPGGYGPGQQGPGGPGAAAAAAGPGGYGPGQQGTG
AAAAAAAGSGAGGYGPGQQGPGGPGAAAAAAGPGGYGPGQQGPGAAAAAAAGSGPGGYGP
GQQGPGGPGAAAAAAAGRGPGGYGPGQQGPGGS

SEQ ID NO: 43
Amino acid sequences encoded by SEQ ID NO: 15

QGPSGPGSAAAAAAAGPGQQGPGGYGPGQQGPGGYGPGQQGPSGPGSAAAAAAAAAAGPG
QQGPGGYGPGPQGPGGYGPGQQGPSGYGPGQQGPSGPGSAASAAAAAGSGQQGPGGYGPG
QQGPGGYGPGQQGPSGPGSAAAAAAAGPGQQGPGGYGPGQQGPGGYGPGQQGPSGPGSAA
AAAAAAGPGQQGPGGYGPGQQGPGGYGPGQQGPSGPGSAAAAAAAGPGQQGPGGYGPGQQ
GPGGYGPGQQGPSGPGSAAAAAAAAAGPGQQGPGGYGPGQQGPGQQGPSGPGSAAAAAAA
GPGPQGPGGYGPGQQGPGGYGPSGPGSAAAAAAAAGPGQQGPGGYGPGQQRPSGYGPGQQ
GPSGPGSAAAAAAAGPGQQGPGAYGPSGPGSAAAAAGLGGYGPAQQGPSGAGSAAAAAAA
GPGGYGPVQQGPSGPGSAAGPGGYGPAQQGPARYGPGSAAAAAAAAGSAGYGPGPQASAA
ASRLASPDSGARVASAVSNLVSSGPTSSAALSSVISNAVSQIGASNPGLSGCDVLIQALL
EIVSACVTILSSSSIGQVNYGAA

SEQ ID NO: 44
Amino acid sequences encoded by SEQ ID NO: 16

QGPGGYGPSGPGSAAAASAAAGPGQQGPGAYGPSGPGSAAAAAGPGXYGPGQQGPSGPGA
AAAAAGPGQQGPGGYGPGAAAAAAAAAGPGQQGPVAYGPSGPGSAASAAGPGGYGPARYG
PSGSAAAAAAAGAGSAGYGPGPQASAAASRLASPDSGARVASAVSNLVSSGPTSSAALSS
VIXNAVSQIGASNPGLSGCDVLIXALLEIVSACVTILSSSSIGQVNYGAA

SEQ ID NO: 45
Amino acid sequences encoded by SEQ ID NO: 17

AGGPGAGGAGAGGVGPGGFGGPGGFGGAGGPGGPGGPGGAGGGAGGAGGLYGPGGAGGL
YGPGGLYGPGGAGVPGAPGASGRAGGIGGAAGGAGAGGVGPGGVSGGAGGAGGSGVTVV
ESVSVGGAGGPGAGGVGPGGVGPGGVGPGGIYGPGGAGGLYGPGAGGAFGPGGGAGAPG
GPGGPGGPGGPGGLGGGVGGAGTGGGVGPGAGGVGPSGGAGGTGPVSVSSTVSVGGAGG
PGAGGPGAGGAGAGGVGPGGFGGPGGFGGAGGPGGPGGPGGAGGGAGGAGGLYGPGGAG
GLYGPGGLYGPGGAGVPGAPGASGRAGGIGGAAGAGGVGPGGVSGGAGGSGVSVTESVT
VGGAGGAGAGGIGGPSGLGGAGATGGFGGRGGPGGPGGPGGPGRFGGAAGGAGAGGVGP
GGVSGGAGGAGGSGVTVVESVSVGGAGGPGAGGVGPGGVGPGGVGPGGIYGPGGAGGLY
GPGAGGAFGSGGGAGAPGGPGGPGGPGGPGGLGGGVGGAGTGGGVGPGVGGVGPSGGAG
GTGPVSVSSTITVGGGQSSGGVLPSTSYAPTTSGYERLPNLINGIKSSMQGGGFNYQNF
GNILSQYATGSGTCNYYDINLLMDALLAALHTLNYQGASYVPSYPSPSEMLSYTENVRR
YF

SEQ ID NO: 46
Amino acid sequences encoded by SEQ ID NO: 18

GAPGGGPGGAGPGGAGFGPGGGAGFGPGGGAGFGPGGAAGGPGGPGGPGGPGGAGGYGPG
GAGGYGPGGVGPGGAGGYGPGGAGGYGPGGSGPGGAGPGGAGGEGPVTVDVDVTVGPEGV
GGGPGGAGPGGAGFGPGGGAGFGPGGAPGAPGGPGGPGGPGGPGGPGGVGPGGAGGYGPG
GAGGVGPAGTGGFGPGGAGGFGPGGAGGFGPGGAGGFGPGGAGGYGPGGVGPGGAGGFGP
GGVGPGGSGPGGAGGEGPVTVDVDVSVGGAPGGGPGGAGPGGAGFGPGGGAGFGPGGGAG
FGPGGAAGGPGGPGGPGGPGGAGGYGPGGAGGYGPGGVGPGGAGGYGPGGAGGYGPGGSG
PGGAGPGGAGGEGPVTVDVDVTVGPEGVGGGPGGAGPGGAGFGPGGGAGFGPGGAPGAPG
GPGGPGGPGGPGGPGGVGPGGAGGYGPGGAGGVGPAGTGGFGPGGAGGFGPGGAGGFGPG
GAGGFGPAGAGGYGPGGVGPGGAGGFGPGGVGPGGSGPGGAGGEGPVTVDVDVSVGGAPG
GGPGGAGPGGAGFGPGGGAGFGPGGGAGFGPGGAAGGPGGPGGPGGPGGAGGYGPGGAGG
YGPGGVGPGGAGGYGPGGAGGYGPGGSGPGGAGPGGAGGEGPVTVDVDVTVGPEGVGGGP
GGAGPGGAGFGPGGGAGFGPGGAPGAPGGPGGPGGPGGPGGPGGVGPGGAGGYGPGGAGG
FGPGGTGGFGPGGAGGFGPGGAGGFGPGGAGGFGPGGAGGYGPGGVGPGGAGGFGPGGVG
PGGSGPGGAGGEGPVTVDVDVSVGGAPGGGPGGAGPGGAGFGPGGGAGFGPGGGAGFGPG
GAAGGPSGPGGPGGPGGAGGYGPGGAGGYGPGGVGPGGAGGYGPGGAGGYGPGGSGPGGA
GPGGAGGEGPVTVDVDVTVGPEGVGGGPGGAGPGGAGFGPGGGAGFGPGGAPGAPGGPGG
PGGPGGPGGPGGVGPGGAGGYGPGGAGGVGPAGTGGFGPGGA

SEQ ID NO: 47
Amino acid sequences encoded by SEQ ID NO: 19

GSGQGRYGGQGSSGGYGQGAGAGAATAATARADGSGQGRYDGQSSQGGYGQGAGAGATA
TAAAGGAGSGQGGYGGQGGLGGYGQGAGAGAAAATAAGGAGSGQGDYGDQGGLGGYGQG
SGAGSATAPAAGGSGFGQGGFGNRGGKGAYGQSAGAGVGAAATAAAGGAGSGQGGYGDQ
GGLGGYGQGAGAGAASAAAGGGDGYEQGGYGNQGGLGSFGQGAGAGAAAAASAGGAGSG
RGGYGDQGGLGGYGQGAGAGAASAAAGGGDGYGQGYYGDQGGRGGYGQGSGAGSATAAA
AGGAGFGQGGYGQGGYGNQGGLGSFGQGAGAGAAAAASAGGAGSGRGGYGDQGGLGGYG
QGAGAGAAAAAAGGGDGYGQGGYGNQGGLGSFGQGAGAGAAAAASAGGAGSGRGGYGQG
GYGNQGGLGSFGQGAGAGAAAAASAGGAGSGRGGYGDQGGLGGYGQGAGSGAAAAAAGG
GDGYGQGGYGNQGGLGSFGQGAGAGAAAAASAGGAGSGRGGYGQGGYGNQGGLGSFGQG
AGAGAAAAASAGGAGSGRGGYGDQGGLGGYGQGAGAGAASAAAGGGDGYGQGGYGNQRG
VGSYGQGAGAGAAATSAAGGAGSGRGGYGEQGGLGGYGQGAGAGAASTAAGGGDGYGQG
GYGNQGGRGSYGQGSGAGAGAAVAAAAGGAVSGQGGYDGEGGQGGYGQGSGAGAAVAAA
SGGTGAGQGGYGSQGSQAGYGQGAGFRAAAATAAAGAGGAGGGQGGYGGQGGYGQGTGA
GGASSAGLSVTVGNMVSRLSSPEAASRVSSAVSSLVSNGQVNVDALPSIISNLSSSISA
SATTASDCEVLVQVLLEVVSALVQIVCS

SEQ ID NO: 48
Amino acid sequences encoded by SEQ ID NO: 20

YGQGSGAGAAAAAAAAGGAGQSGSGPYGASYLSSTTYTTSSQGAGGGVGGYGQGSGTGSA
AAAAGAAGAGQGGQGGYGQGAGQGGLGGYGQGGGAGAAAAAAAAAGGAGSGQGGYGGQGG
LGGYGQGAGAGAAAAAAAGGAGAGQGGFGGQGGYGQGGGAGAAAAAAAAAAAGGAGSGQG
GYGGQGGLGGYGQGAGAGAAAAAAAGGAGAGQGSYGGQGGYGQGGAGAATATAAAAGGAG
SGQGGYGGQGGLGGYGQGAGAGAAAAAAAAAGGAGAGQGGYGGQGGQGGYGQGAGAGAAA
AAAGGAGAGQGGYGGQGGYGQGGGAGAAAAAAAAASGGSGSGQGGYGGQGGLGGYGQGAGA
GAGAAASAAAAGAGSGQGGYGGQGGLGGYGQGAGAGAAAGASGSGSGGAGQGGLGGYGQG
AGAGAAAAAAGASGAGQGGFGPYGSSYQSSTSYSVTSQGAAGGLGGYGQGSGAGAAAAGA
AGQGGQGGYGQGAGAGAGAGAGQGGLGGYGQGAGSSAASAAAAGGAGAGQGGYGGQGGLG
GYGQGAGAGASAAASASGAGSGQGGYGGQGGYGQGTGAGAASSAGVAVTVGNTVSRLSSP
QAASRVSSAVSSLVSNGQVNVAALPSIISSLSSSISASSTAASDCEVLVQVLLEIVSALV
QIVSSANVGYINPEASGSLNAVGSALAAAMG

SEQ ID NO: 49
Amino acid sequences encoded by SEQ ID NO: 21

NASQIAASVASAVASSASAAAAAASSSAAAAAGASSAAGAASSSSTTTTTSTSSSAAAA
AAAAAAASASGASSASAAASASAAASAFSSALISDLLGIGVFGNTFGSIGSASAASSIA
SAAAQAALSGLGLSYLASAGASAVASAVAGVGVGAGAYAYAYAIANAFASILANTGLLS
VSSAASVASSVASAIATSVSSSSAAAAASASAAAAASASAASSASASSSASAAAAAGAS
AAAGAASSASASAAASAFSSAFISALLGFSQFNSVFGSITSASLGLGIAANAVQSGLAS
LGLGAAASAAASAVANAGLNGSGAYAYATAIASAIGNALLGAGFLTAGNASQIAASVAS
AVASSASAAAAAAASSSAAAAGASSAAGAASSSSTTTTTSTSSSAAAAAAAAAAASASGA
SSASAAASASAAASAFSSALIGDLLGIGVFGNTFGSIGSASAASSIASAAAQAALSGLG
LSYLASAGASAVASAVAGVGVGAGAYAYAYAIANAFASILANTGLLSVSSAASVASSVA

SAIATSVSSSSAAAAASASAAAAASAGASAASSASASSSASAAAGAGAGAGAGASGASG
AAGGSGGFGLSSGFGAGIGGLGGYPSGALGGLGIPSGLLSSGLLSPAANQRIASLIPLI
LSAISPNGVNFGVIGSNIASLASQISQSGGGIAASQAFTQALLELVAAFIQVLSSAQIG
AVSSSSASAGATANAFAQSLSSAFAG

SEQ ID NO: 50
Amino acid sequence encoded by SEQ ID NO: 22

AAAAAAAAAAGAGAGAGAGAGAGAGAGSGASTSVSTSSSSGSGAGAGAGSGAGSGAGAG
SGAGAGAGAGGAGAGFGSGLGLGYGVGLSSAQAQAQAQAAAQAQAQAQAQAYAAAQAQA
QAQAQAQAAAAAAAAAAAGAGAGAGAGAGAGAGAGSGASTSVSTSSSSGSGAGAGAGSG
AGSGAGAGSGAGAGAGAGGAGAGFGSGLGLGYGVGLSSAQAQAQAQAAAQAQAQAQAQA
YAAAQAQAQAQAQAQAAAAAAAAAAAAGAGAGAGAGAGAGAGAGSGASTSVSTSSSSGSG
AGAGAGSGAGSGAGAGSGAGAGAGAGGAGAAFGSGLGLGYGVGLSSAQAQAQAQAAAQA
QADAQAQAYAAAQAQAQAQAQAQAAAAAAAAAAAAGAGAGAGAGSGAGAGAGSGASTSVS
TSSSSGSGAGAGAGSGAGSGAGAGSGAGAGAGAGGAGAGFGSGLGLGYGVGLSSAQAQA
QAQAAAQAQADAQAQAYAAAQAQAQAQAQAQAAAAAAAAAAAAAGAGAGAGAGSGAGAGAG
SGASTSVSTSSSSGSGAGAGAGSGAGSGAGAGSGAGAGAGAGGAGAGFGSGLGLGYGVG
LSSAQAQAQSAAAARAQADAQAQAYAAAQAQAQAQAQAQAAAAAAAAAAAAGAGAGAGAG
AGAGAGAGSGASTSVSTSSSSASGAGAGAGSGAGSGAGAGSGAGAGAGAGGAGAGFGSG
LGLGYGVGLSSAQAQAQAQAAAQAQAQAQAQALAAAQAQAQAQAQAQAAAATAAAAAAG
AGAGAGSGAGAGAGAGAGSGASTSVSTSSSSAAGAGAGAGSGAGAGSGTGAGIALPSIV
LSPAASSRISSVSSSVQSAGSGLSFSSLSNTLSQTASAIRSSNPQLSSSDVLIQSLVEI
IVGLVQAFTGSSASAQTFVNSLSQVAG

SEQ ID NO: 51
Amino acid sequence encoded by SEQ ID NO: 23

TDSVASSASSSASASSSATGPDTGYPVGYYGAGQAEAAASAAAAAAASAAEAATIAGLGY
GRQGQGTDSSASSVSTSTSVSSLATGPGSRYPVRDYGADQAEAAASAAAAASAAEEIASL
GYGRQGQGTDSVASSASSSASASSSATGPDTGYPVGYYGAGQAEAAASAAAAAAASAAEA
ATIAGLGYGRQGQGTDSSASSVSTSTSVSSSATGPDTGYPVGYYGAGQAEAAASAAAAAA
ASAAEAATIAGLGYGRQGQGTDSSASSVSTSTSVSSSATGPDMGYPVGNYGAGQAEAAAS
AAAAAAASAAEAATIASLGYGRQGQGTDSSASSVSTSTSVSSSATGPGSRYPVRDYGADQ
AEAAASAAAAAAAAASAAEEIASLGYGRQGQGTDSVASSASSSASASSSATGPDTGYPVG
YYGAGQAEAAASAAAAAAASAAEAATIAGLGYGRQGQGTDSSASSVSTSTSVSSSATGPG
SRYPVRDYGADQAEAAASATAAAAAAASAAEEIASLGYGRQGQGTDSVASSASSSASASS
SATGPDTGYPVGYYGAGQAEAAASAAAAAAASAAEAATIAGLGYGRQGQGTDSSASSVST
STSVSSSATGPGSRYPVMDYGADQAEAAASAAAAAAAEAATIAGLDYEGQGQGTDSGASS
VSSSTSVSSSATGVTQTTIALPPDVSARISFLTSYLQSAGSGLSLYTLSNLLSQTALAIS
KSRPELSPNEVLIQSLAEIIVALVQALTKQASSSASVQYFGRFL

SEQ ID NO: 52
Amino acid sequence encoded by SEQ ID NO: 24

AISSSLYAFNYQASAASSAAAQSSAQTASTSAKQTAASTSASTAATSTTQTAATTSASTA
ASSQTVQKASTSSAASTAASKSQSSSAGSSRTTSTAAASASSSYAFAQSLSQYLLSSQQF

TTAFASSTAVASSQQYAEAMAQSVATSLGLGYTYASALSVAMAQAISGVGGGASAYSYAT
AISQAISRALTSSGVSLSSSQATSVASAISSSLYAFNYQASAASSAAAQSSAQTASTSAK
QTAASTSASTAATSTTQTAATTSASTAASSQTVQKASTSSAASTAASKSQSSSVGSSTTS
TAAASASSSYAFAQSLSQYLLSSQQFTTAFASSTAVASSQQYAEAMAQSVATSLGLGYTY
TSALSVAMAQAISGVGGGASAYSYATAISQAISRVLTSSGISLSSSQATSVASAISSSLY
AFNYQASAASSAAAQSSAQTASTSAKQTAASTSASTAATSTTQTAATTSASTAASSQTVQ
KASTSSAASTAASKSQSSSVGSSTTSTAAASASSSYAFAQSLSQYLLSSQQFTTAFASST
AVASSQQYAEAMAQSVATSLGLGYTYTSALSVAMAQAISGVGGGASAYSYATAISQAISR
VLTSSGVSLSSSQATSVASAISSSLYAFNYRASAASSAAAQSSAQTASTSAKQTAASTSA
STAATSTTQTAATTSASTAASSQTVQKASTSSAASTAAQQTGQSSSVQNQGSSSASSSSV
SVSDISDSLTTSLLQSEEFTSAFGSTVSEAEAQSYAEAVAQSTVAQLGIDYSQSSALATA
VANAVSQVKQGSSSRAYARAIAYAITTYLKTTRIITTITRTQVKSFASAISSSLSTARAT
SSANAYQEQTTQSSAAASAAAQSSEYQTQNTQSSASAASSDASTSYQTQQSYSDASAASV
AAESTSANQAQSTQSSAAASSSTNSAYQSQQSYIDASTVSSASANTAQSTYQVTIPDNTY
FAESLSSTLIQHEQFNSKFGSYIPLVTAREYASAMARATALIIGFDSTGTSALESAVAVA
VSNVDYASAYSYARAIAFAISNVLTNNGIFASASEALYLAPAMIASLHAFGKSSFSESSA
FALANSISPSTAITSAQSSSVSAGASSGQSSYDTSSVVSSASSAEATESSSVFDTYQATQ
IESSAAAAAASSSAYDSQFSESSSASSAAASAFSEQTSYDISSDLSSASSATAAAASSSA
YESQFSDASSGSSAAAAASSQQNSYDTDALYSASSAASAAASASAYELEFSDASSSSSAV
AVASSQQGSYDTSSDFSSASSAAAAAASAYESKFLDASSSSSAAAAASSQQSSYETSSDL
VSASSAAAAAASASAYQSQFLDASSSSNAAATTSSRQSSYDTSSDFSSASIAAAAAASAS
SYESQFSDASSSSNAAAAASSQQSSYDTSSDLVSAASASAYESQFLDASSSSNAAATTSS
QQSSYDTSSDFSSASIAAAAAASASSYESQFSDASSSSNAAAAASSQQSSYDTSSDLVSA
SSAAAAAASASSYESQFSDASSSSNAAAAASSQQSSYDTSSDLVSASSAAAAAASASAYE
SQFSDASSSRNAAAAASSQQSSYDTSSDLVSASSAAAAAASASSYESQFLDASSSSNAAA
TTSSQQSSYDTSSDFSSASIAAAVAASASSYESQFSDASSSSKAAAAASSQQSSYDTSSD
LVSASSAAAAAASASSYESQFSDASSSSNAAAAASSQQSSYDTSSDFSSANSAALAESSA
ATEIYQETQIASSIAAASALSEAHTSELAEASSSSSAASAAAAAASEQSLYDTSSAASSA
SSSDFIASSDIRNQQSLSVNSAASSSAAEESVSQVDEETYQNFDQYSSISASASAAQSSE
IYQDVSSSSAAASTSSAASSLETSGTVAESGSTAASSSYAAAAAASSSAGSTSSPSFLSA
DSLSSSLASLRICSFSSKLMSSLYSGDGLDIAEFSDAVSSMVSSIKSSNPGVSASQILTE
LLFEVIVAFVQALTKSKFSTMETAESLIAAFAQAFV

SEQ ID NO: 53
Amino acid sequence encoded by SEQ ID NO: 25

SQQGPIGGVGGSNAFSSSFASALSLNRGFTEVISSASATAVASAFQKGLAPYGTAFALSA
ASAAADAYNSIGSGANAFAYAQAFARVLYPLVRQYGLSSSGKASAFASAIASSFSSGTSG
QGPSIGQQQPPVTISAASASAGASAAAVGGGQVGQGPYGGQQQSTAASASAAAATATSGA
AQKQPSGESSVATASAAATSVTSGGAPVGKPGVPAPIFYPQGPLQQGPAPGPSNVQPGTS
QQGPIGGVGGSNAFSSSFASALSLNRGFTEVISSASATAVASAFQKGLAPYGTAFALSAA
SAAADAYNSIGSGANAFAYAQAFARVLYPLVRQYGLSSSGKASAFASAIASSFSSGTSGQ
GPSIGQQQPPVTISAASASAGASAAAVGGGQVGQGPYGGQQQSTAASASAAAATATSGAA
QKQPSGESSVATASAAATSVTSGGAPVGKPGVPAPIFYPQGPLQQGPAPGPSNVQPGTSQ
QGPIGGVGGSNAFSSSFASALSLNRGFTEVISSASATAVASAFQKGLAPYGTAFALSAAS
AAADAYNSIGSGANAFAYAQAFARVLYPLVQQYGLSSSAKASAFASAIASSFSSGTSGQG
PSIGQQQPPVTISAASASAGASAAAVGGGQVGQGPYGGQQQSTAASASAAAATATSGGAQ

KQPSGESSVATASAAATSVTSAGAPVGKPGVPAPIFYPQGPLQQGPAPGPSNVQPGTSQQ
GPIGGVGGSNAFSSSFASALSLNRGFTEVISSASATAVASAFQKGLAPYGTAFALSAASA
AADAYNSIGSGANAFAYAQAFARVLYPLVQQYGLSSSAKASAFASAIASSFSSGTSGQGP
SNGQQQPPVTISAASASAGASAAAVGGGQVSQGPYGGQQQSTAASASAAAATATSGGAQK
QPSGESSVATASAAATSVTSAGAPGGKPGVPAPIFYPQGPLQQGPAPGPSNVQPGTSQQG
PIGGVGGSNAFSSSFASALSLNRGFTEVISSASATAVASAFQKGLAPYGTAFALSAASAA
ADAYNSIGSGANAFAYAQAFARVLYPLVQQYGLSSSAKASAFASAIASSFSSGTSGQGPS
IGQQQPPVTISAASASAGASAAAVGGGQVGQGPYGGQQQSTAASASAAAATATSGGAQKQ
PSGESSVATASAAATSVTSAGAPVGKPGVPAPIFYPQGPLQQGPAPGPSYVQPATSQQGP
IGGAGRSNAFSSSFASALSGNRGFSEVISSASATAVASAFQKGLAPYGTAFALSAASAAA
DAYNSIGSGANAFAYAQAFARVLYPLVQQYGLSSSAKASAFASAIASSFSSGAAGQGQSI
PYGGQQQPPMTISAASASAGASAAAVKGGQVGQGPYGGQQQSTAASASAAATTATAGGAQ
KHPSGEYSVATASAAATSVTSGGAPVGKPGVPAPIFYPQGPLQQGPAPGPSNVQPGTSQQ
GPIGGVGESNTFSSSFASALGGNRGFSGVISSASATAVASAFQKGLAPYGTAFALSAASA
AADAYNSIGSGASASAYAQAFARVLYPLLQQYGLSSSADASAFASAIASSFSTGVAGQGP
SVPYVGQQQPSIMVSAASASAAASAAAVGGGPVVQGPYDGGQPQQPNIAASAAAAATATS
SGPKEEPLGESSVIATSVSAASSVSSGGAPGVQGGGPVTVSYREGPSQIPSQQTLLQAVP
STQSVGSGVPVGPNQYEMVYAPLQQFGGVSASNLLSPSAHSRIASLMSDVLSLFSPGNSG
FNYGGFARALSSVARAVSQSNAKLSTTDVIIQVLMEALVALIELLSGAKIGVVHPVRAQA
GASAFAQHFGSAFG

SEQ ID NO: 54
Amino acid sequences encoded by SEQ ID NO: 26

AGAGAGYGAGAGSGAGAGSGAGAGAGAGAGAGYGAGAGSGAGAGAGYGRGAGAGAGA
GAGYGQGAGAGAGAGAGYGAGAGSGAGAGYGTGAGSGAGSGAGSGAGSGAGAGAGAGAG
YGAGAGSGAGAGAGYGRGAGAGAGAGAGYGQGAGAGAGAGAGAGSGAGAGSGAGAGAGA
GYGQGAGAGAGAGAGYGAGAGSGAGAGAGYGAGAGSGAGAGSGAGAGAGAGYGQGAGAG
AGAGYGAGAGSGAGAGSGAGAGSGAGAGSGYGAGAGSGAGAGAGYGQGAGAGAGAGAGY
GAGAGSGAGTGAGYGAGAGAGYGAGAGAGAGSGAGAGAGYGAGAGAGAGAGYGAGAGSG
XGAGXGYGAGAGAGSGVGAGAGAGAGAGYGAGAGAGAGYGAGAGAGAGAGAGAGYGAGA
GAGASVSSTVSNTASRMSSENTSRRVSSAISSIVGSGGVNMNSLSNVISNVSSSVAASN
PGLSGCEVLVQTLLEVVSALVHILSYASVGSVDASAAGQSAQTVATAMSSVMG*

SEQ ID NO: 55
Amino acid sequence encoded by SEQ ID NO: 27

GAAAAASAAAAGGRGSQGGYGDDGGAAAAAAAAAAAAAAGSGGTGGGQGGRGDGGAAAAA
AAAAEAAAGGKGRQGSYGDDGGAAVAAAAAAAAAAGRGGSGRGQGLRRDKGSYGVDGGAE
AAASAAATAGRQGRQGSYGDDGGAAAAAAAAASASRLASSSAVSRVSSAVSALLSNGFSD
VNSLSNVISGLSASVSSSTPELTGCEVLVEVLLEVVSALVHILNFADIGNVNISASGDST
SLVGRTVLEAFG*

SEQ ID NO: 56
Amino acid sequences encoded by SEQ ID NO: 28

GINVDSGSVQSDISSSSSFLSTSSSSASYSQASASSSSGAGYTGPSGPSTGPSGYPGPL
SGGASFGSGQSSFGQTSAFSASGAGQSAGVSVISSLNSPVGLRSPSAASRLSQLTSSIT
NAVGANGVDANSLARSLQSSFSALRSSGMSSSDAKIEVLLETIVGLLQLLSNTQVRGVN
PATASSVANSAARSFELVLA*

SEQ ID NO: 57
Consensus amino acid sequence of a 200 amino acid repeat unit of SEQ ID NO: 56

SSVVQRAAQSLASTLGVDGNNLARFAVQAVSRLPAGSDTSAYAQAFSSALFNAGVLNAS
NIDTLGSRVLSALLNGVSSAAQGLGINVDSGSVQSDISSSSSFLSTSSSSASYSQASAS
STSGAGYTGPSGPSTGPSGYPGPLGGGAPFGQSGFGGSAGPQGGFGATGGASAGLISRV
ANALANTSTLRTVLRTGVSQQIA